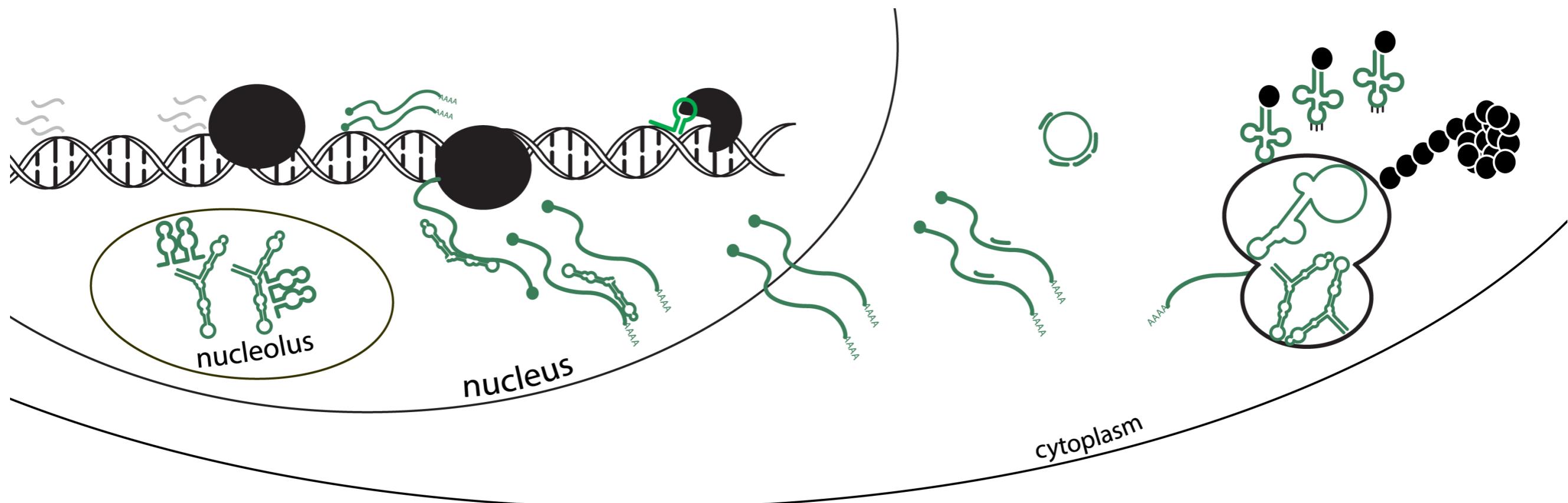
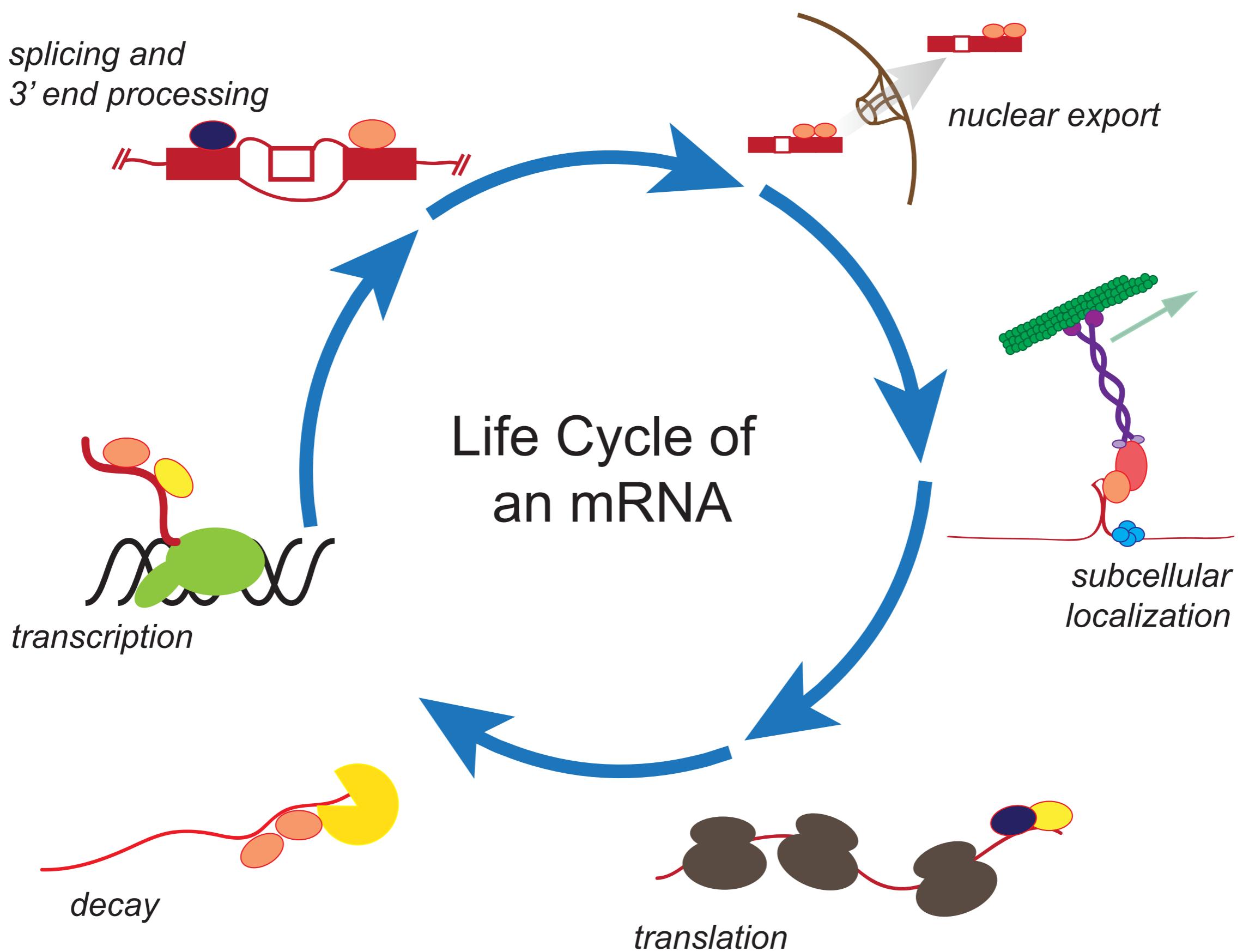


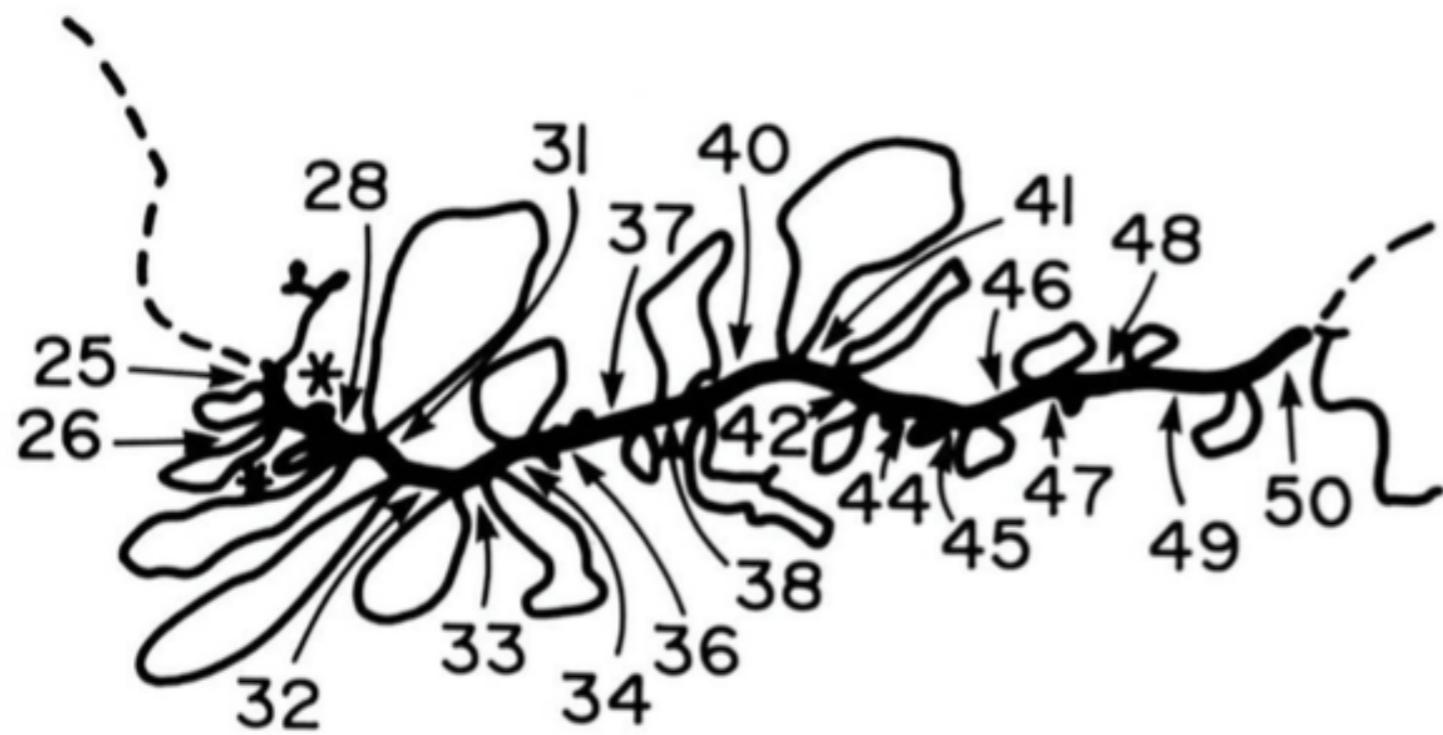
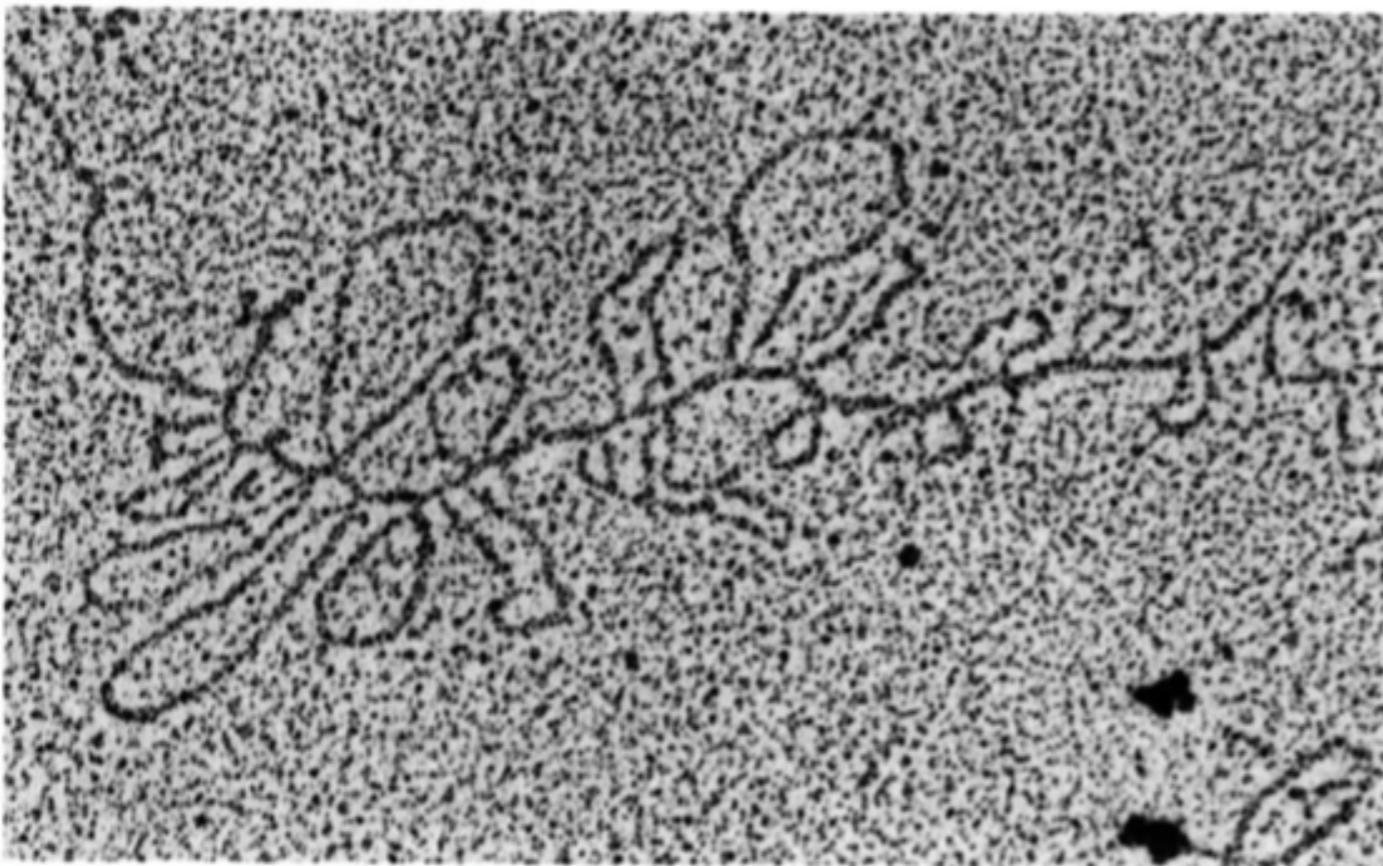
Regulation of gene expression through alternative splicing



mRNA metabolism is highly regulated

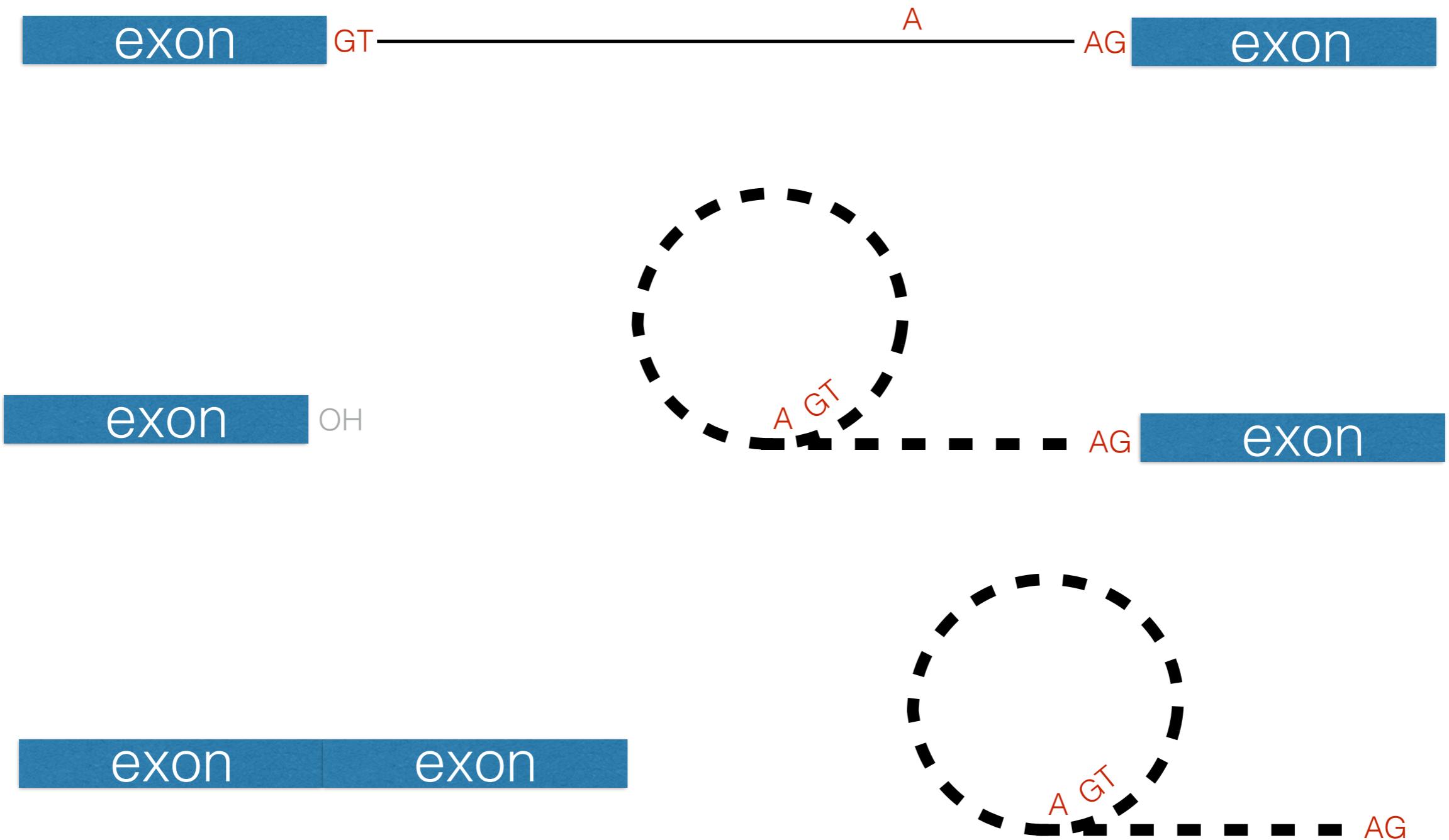


DNA contains sequences that are missing in mRNA

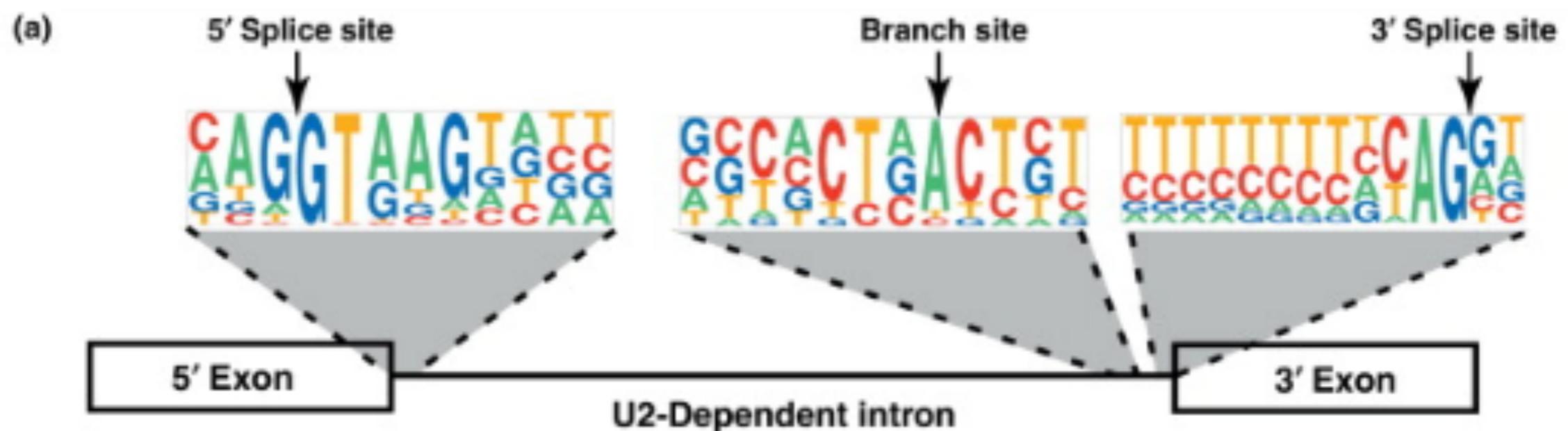


de Wet et al. (1986) PNAS

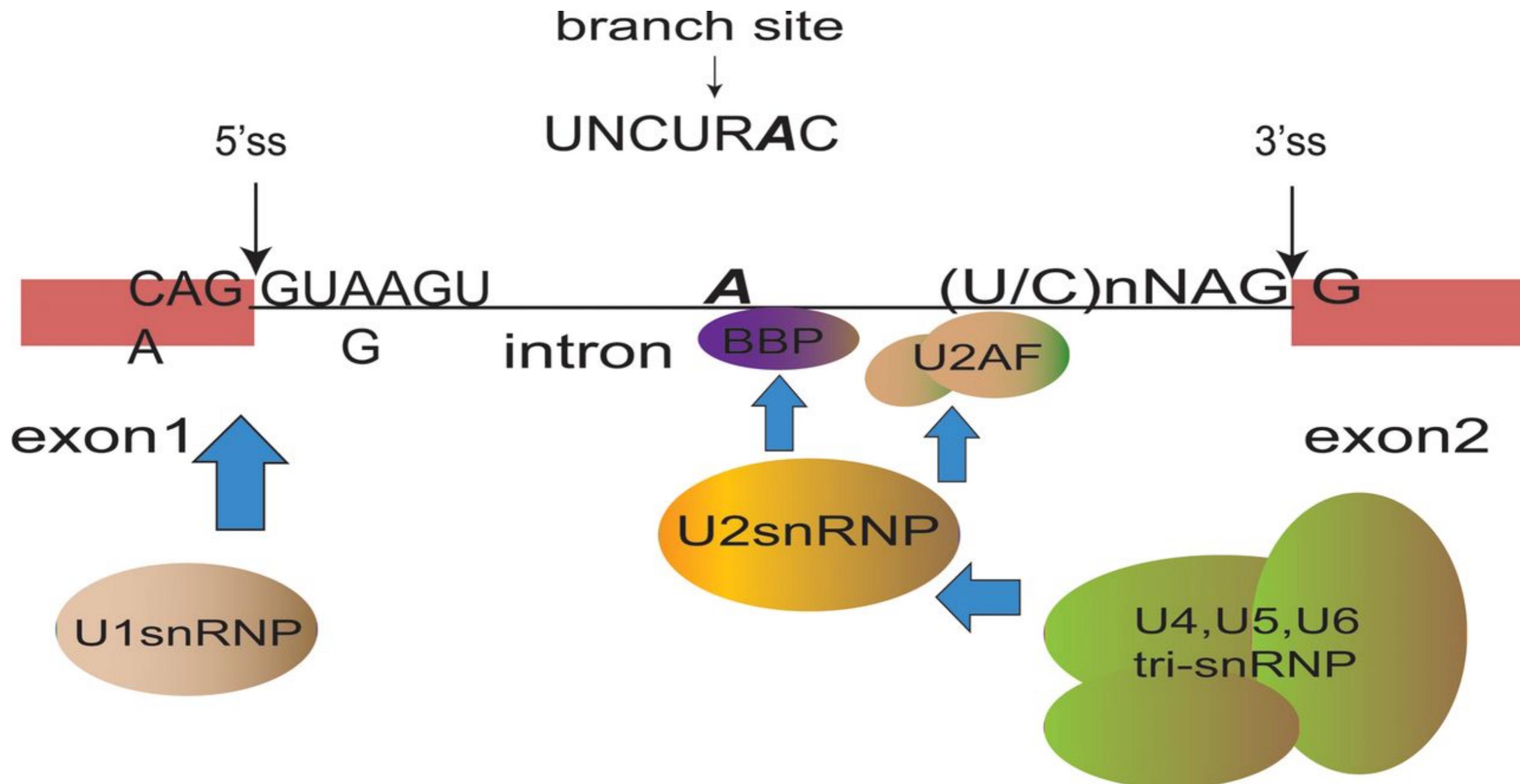
Intronic sequences are removed from pre-mRNA transcripts



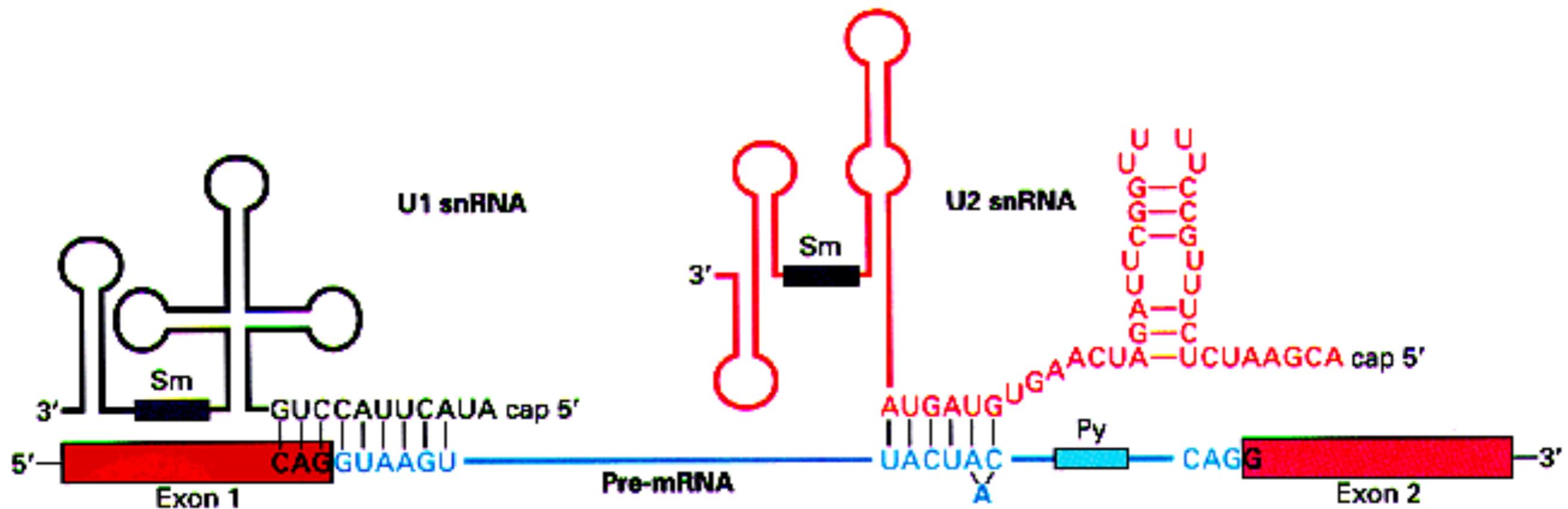
Functional intronic sequences often have characteristic sequences



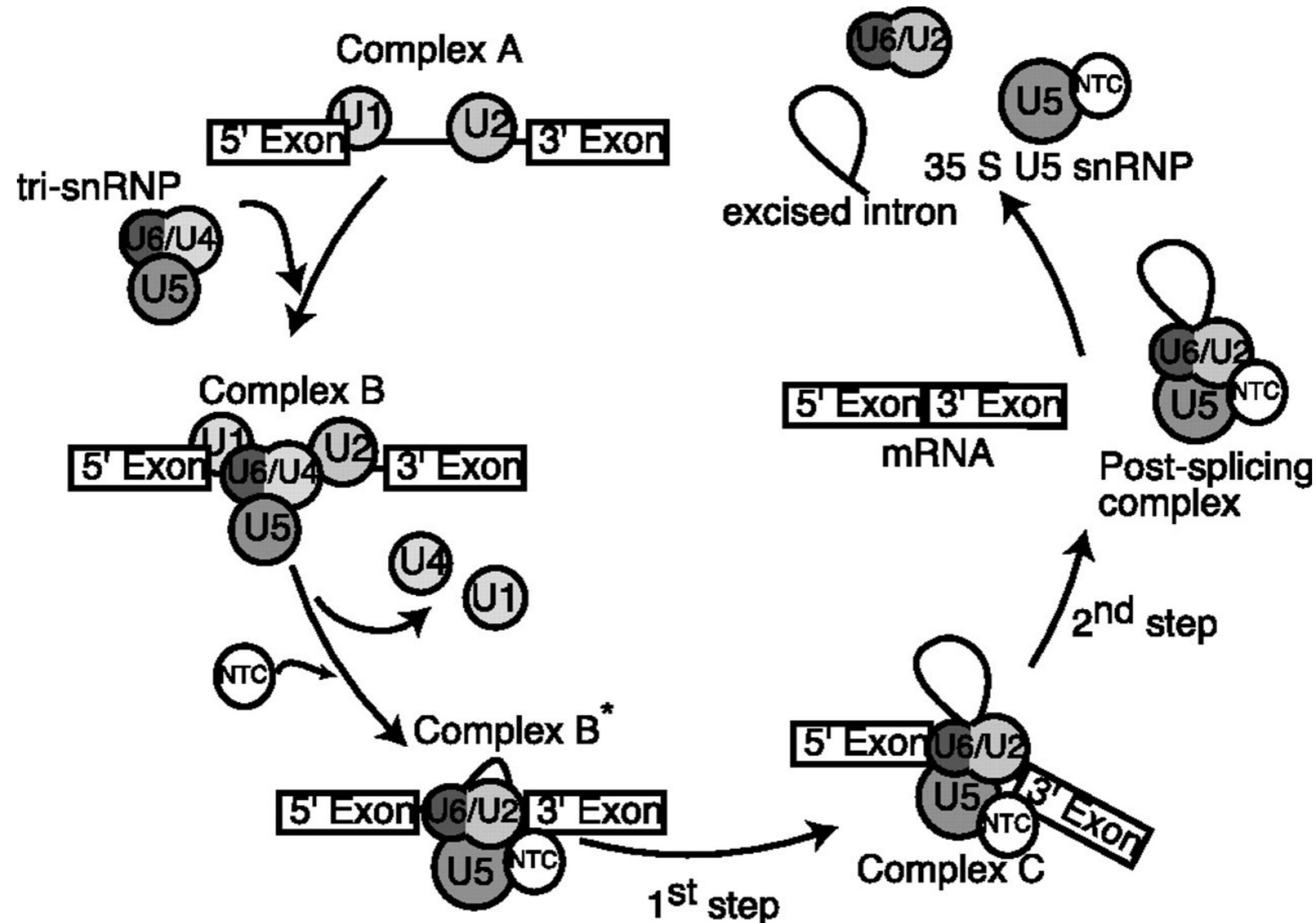
Key intronic regulatory sequences are bound by snRNPs (small nuclear ribonucleoprotein particles)



SNRNPs consist of a small nucleolar RNA (snRNA) and several proteins

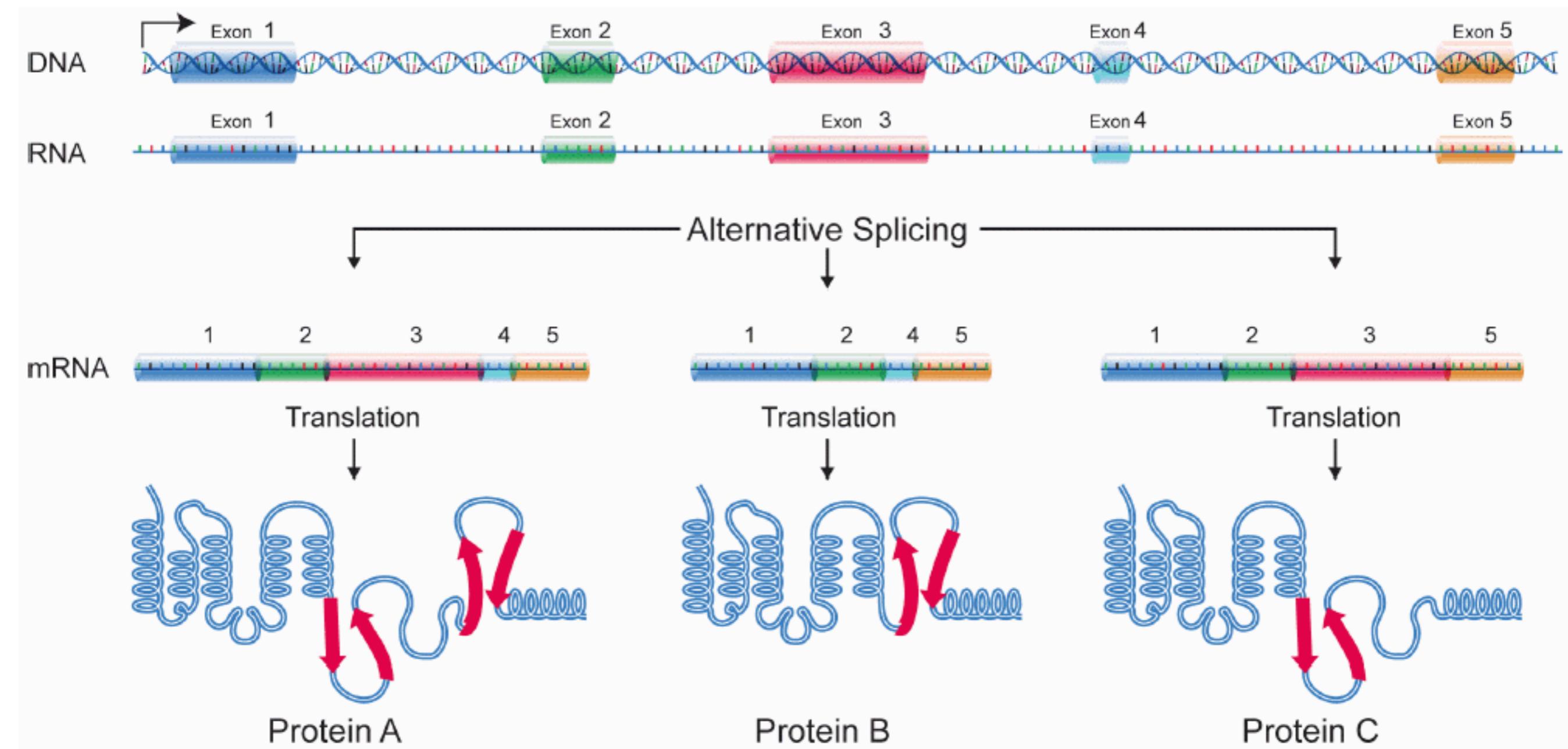


Five snRNPs form the spliceosome

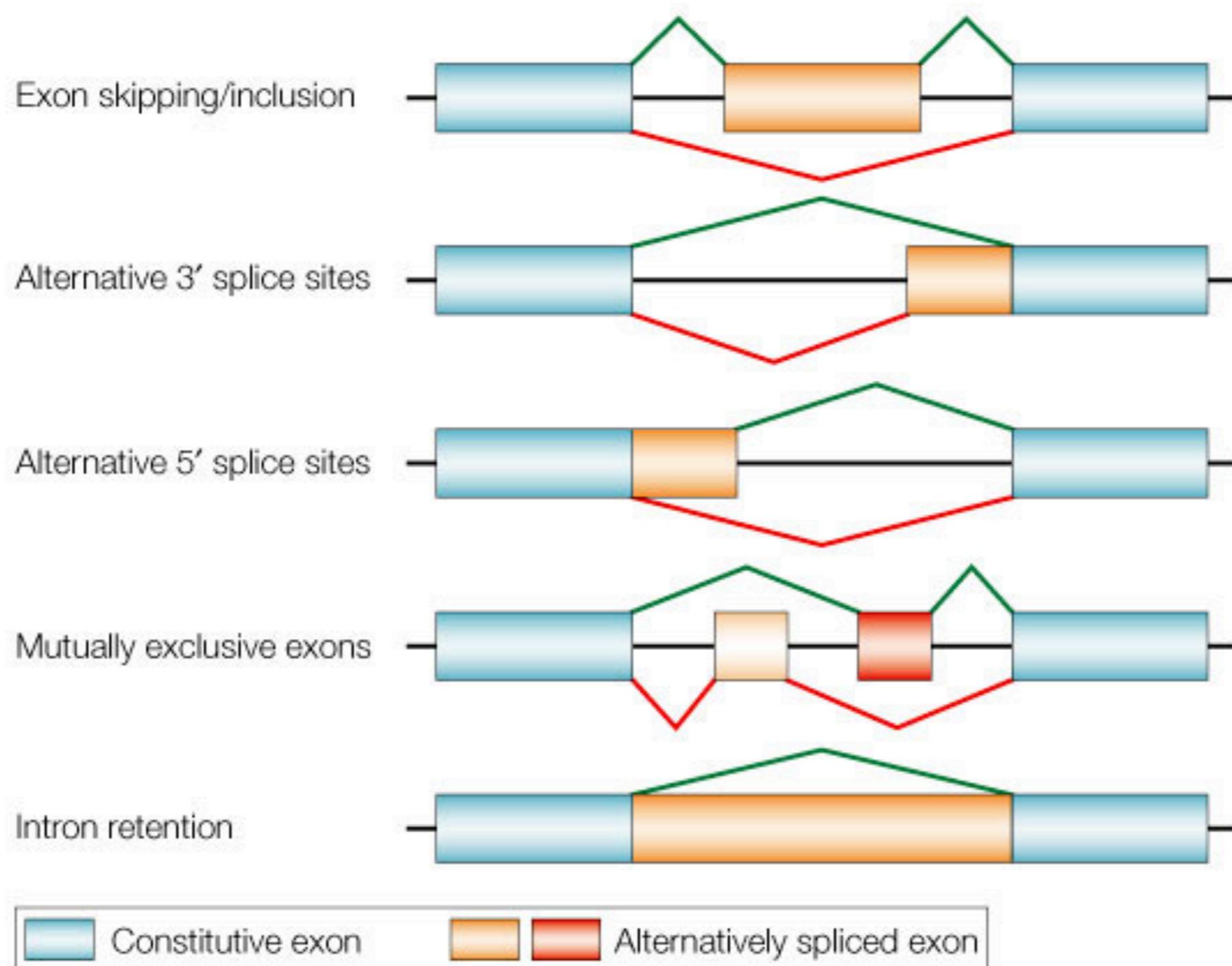


Introns....what are they good for?

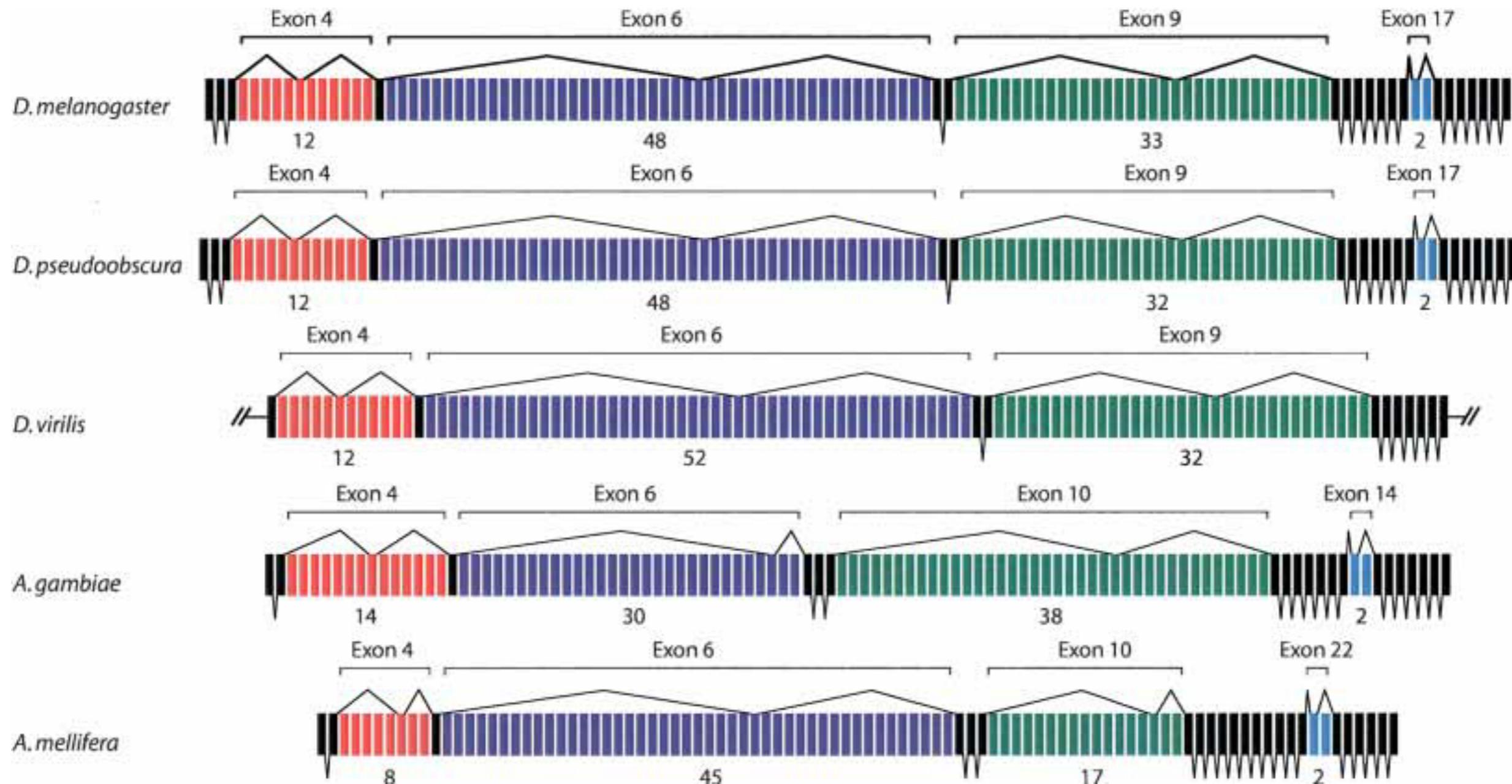
Alternative splicing increases proteomic complexity



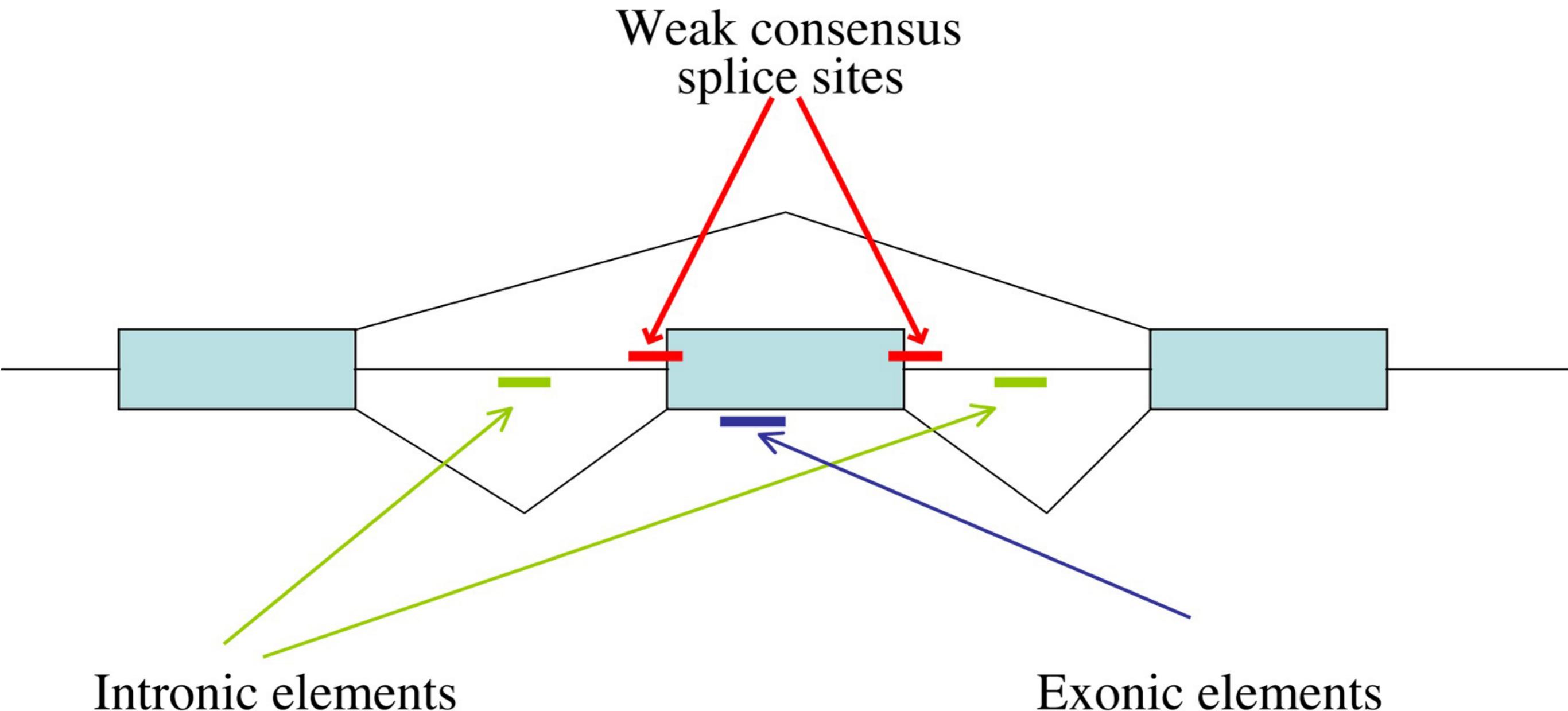
Alternative splicing increases proteomic complexity



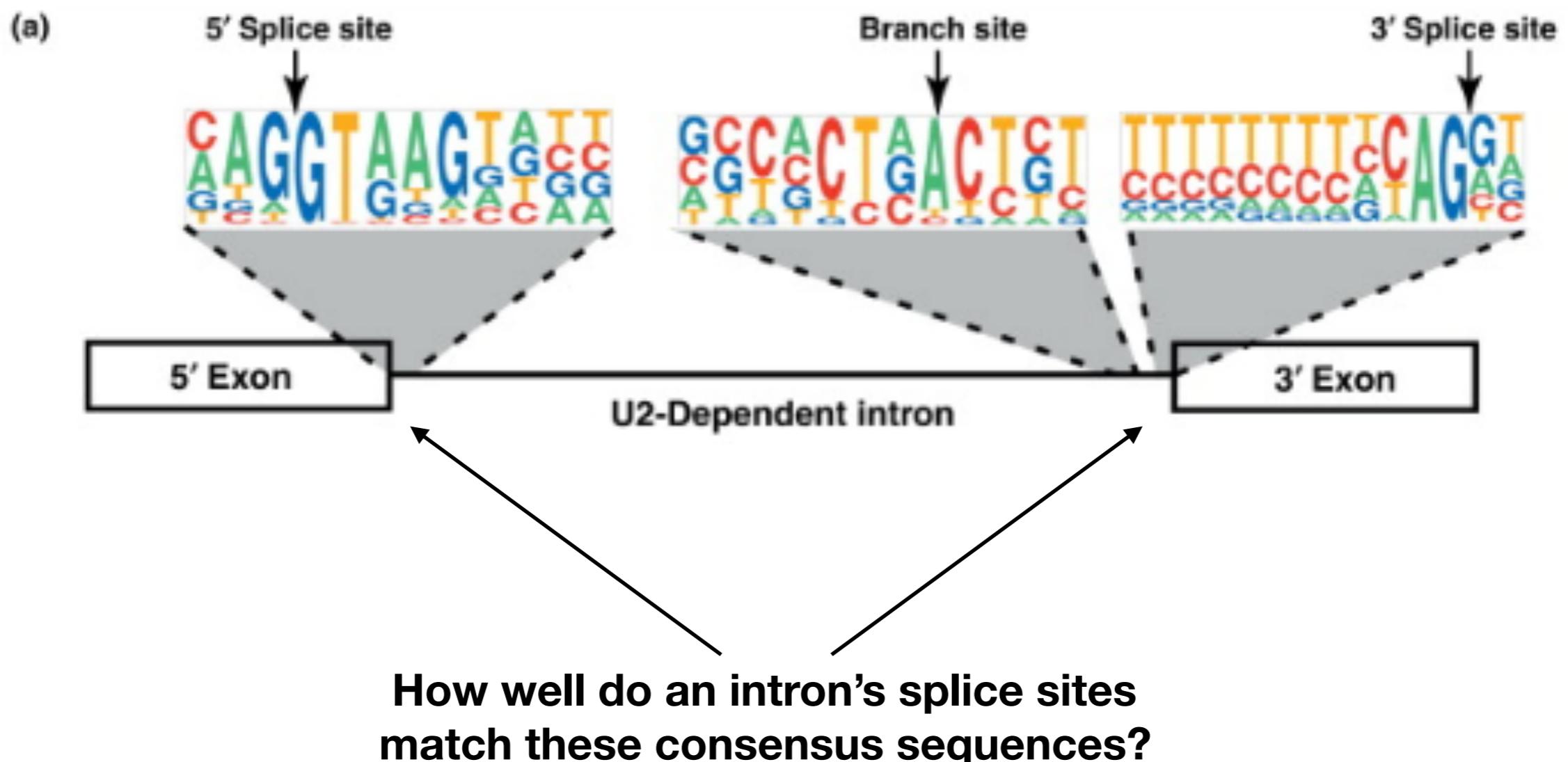
Alternative splicing increases proteomic complexity



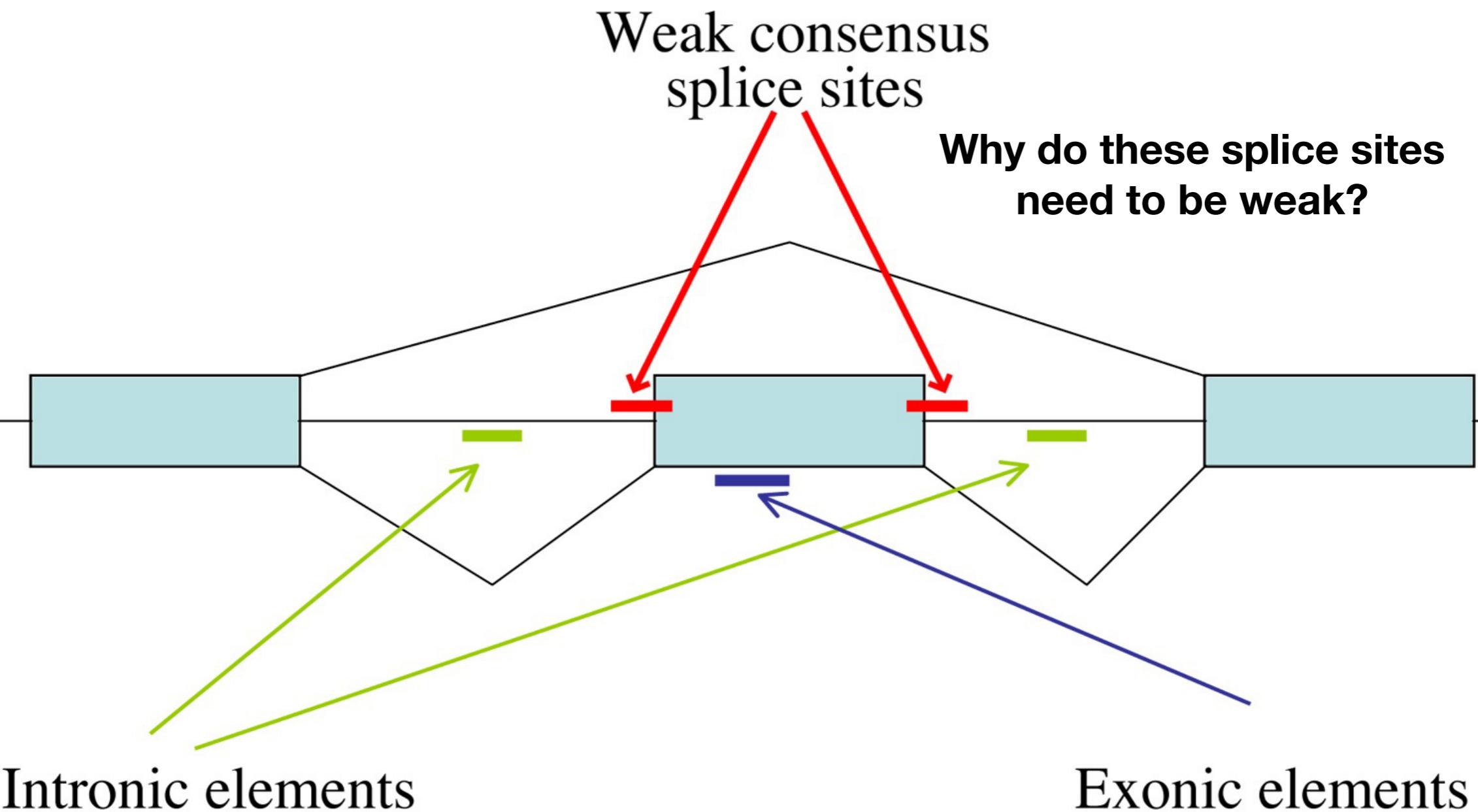
Alternative splicing is regulated through the activity of specific RNA sequence elements



Functional intronic sequences often have characteristic sequences

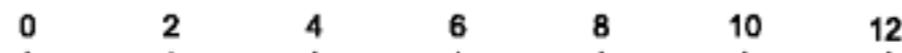


Alternative splicing is regulated through the activity of specific RNA sequence elements



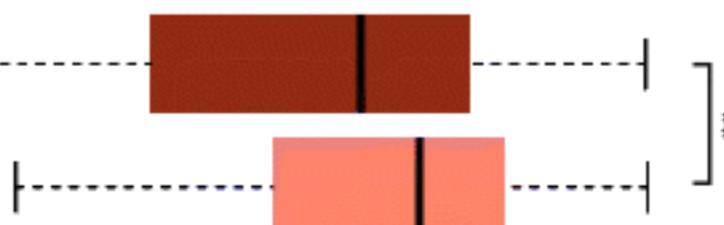
D

5' Splice Site Strength- Differentially Used Exons

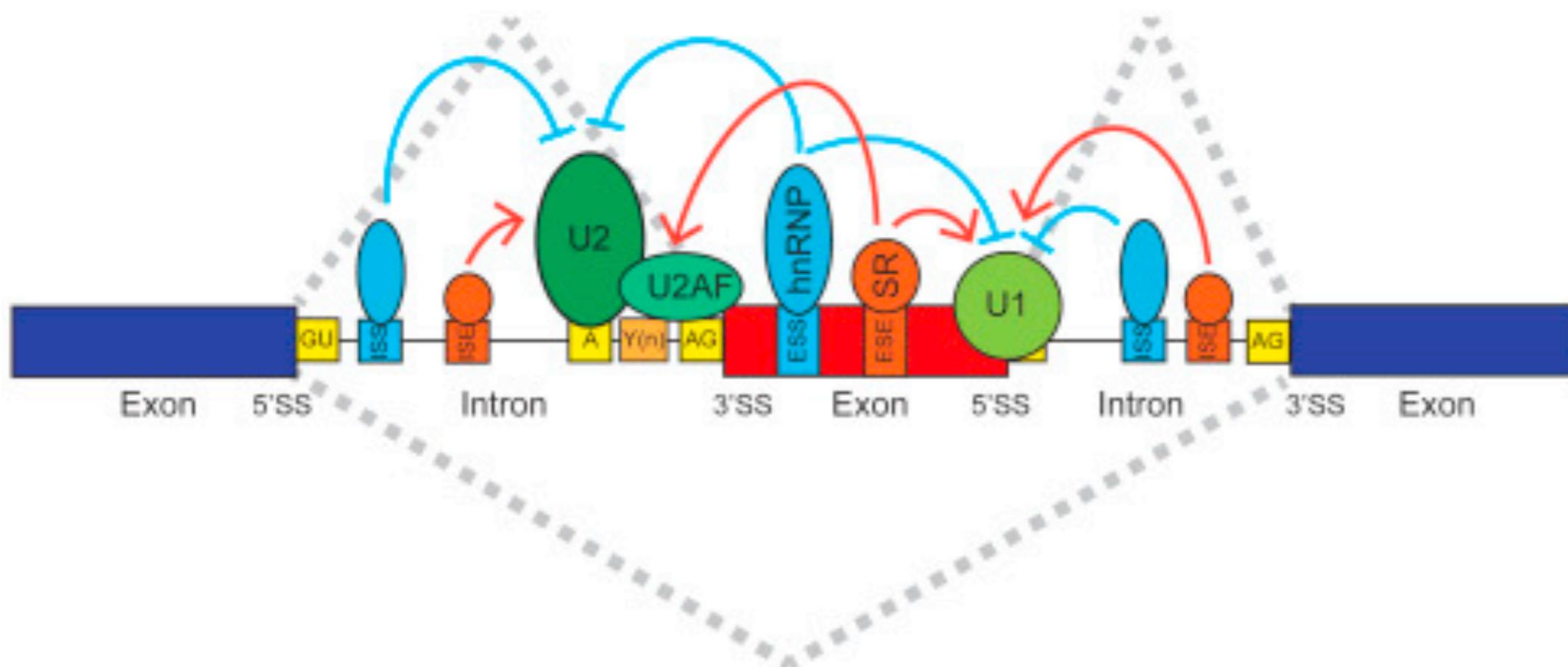


Differentially Used Exons

Control



Alternative splicing is regulated through the activity of specific RNA sequence elements



Many SR proteins can regulate splicing

Table 1 ‘Classical’ SR proteins

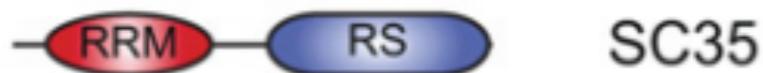
Protein name	Gene name	Key domains	Splicing role	UniProt
SF2/ASF	SFRS1	RRM × 2, RS	Constitutive and alternative splicing activator	Q07955
SC35	SFRS2	RRM, RS	Constitutive and alternative splicing activator	Q01130
SRp20	SFRS3	RRM, RS	Constitutive and alternative splicing activator	P84103
SRp75	SFRS4	RRM × 2, RS	Constitutive and alternative splicing activator	Q08170
SRp40	SFRS5	RRM × 2, RS	Constitutive and alternative splicing activator	Q13243
SRp55	SFRS6	RRM × 2, RS	Constitutive and alternative splicing activator	Q13247
9G8	SFRS7	RRM, RS, CCHC-type zinc finger	Constitutive and alternative splicing activator	Q16629

Table 2 Additional SR proteins

Protein name	Gene name	Key domains	Splicing role	UniProt
p54	SFRS11	RRM, RS	Alternative splicing repressor	Q05519
SRp30c	SFRS9	RRM × 2, RS	Constitutive and alternative splicing regulator	Q13242
SRp38, TASR	FUSIP1	RRM, RS	General splicing repressor	Q75494
hTra2α	TRA2A	RRM, RS × 2	Splicing activator	Q13595
hTra2β	SFRS10	RRM, RS × 2	Splicing activator	P62995
RNPS1	RNPS1	RRM, RS	Constitutive and alternative splicing regulator	Q15287
SRrp35	SRRP35	RRM, RS	Negative regulator of alternative splicing	Q8WXFO
SRrp86, SRrp508	SFRS12	RRM, RS	Positive and negative regulator of alternative splicing	Q8WXA9
U2AF35	U2AF1	RRM, RS, C3H1-type zinc finger × 2	Constitutive splicing factor	Q01081
U2AF65	U2AF2	RRM × 3, RS	Constitutive splicing factor	P26368
U1-70K	SNRP70	RRM, RS	Constitutive splicing factor	P08621
XE7	SFRS17A	RRM, RS	Alternative splicing regulator	Q02040
SRp46	SFRS2B	RRM, RS	Constitutive and alternative splicing regulator	Q9BRL6

SR proteins have one or more Arg/Ser-rich domains and one or more RNA-binding domains

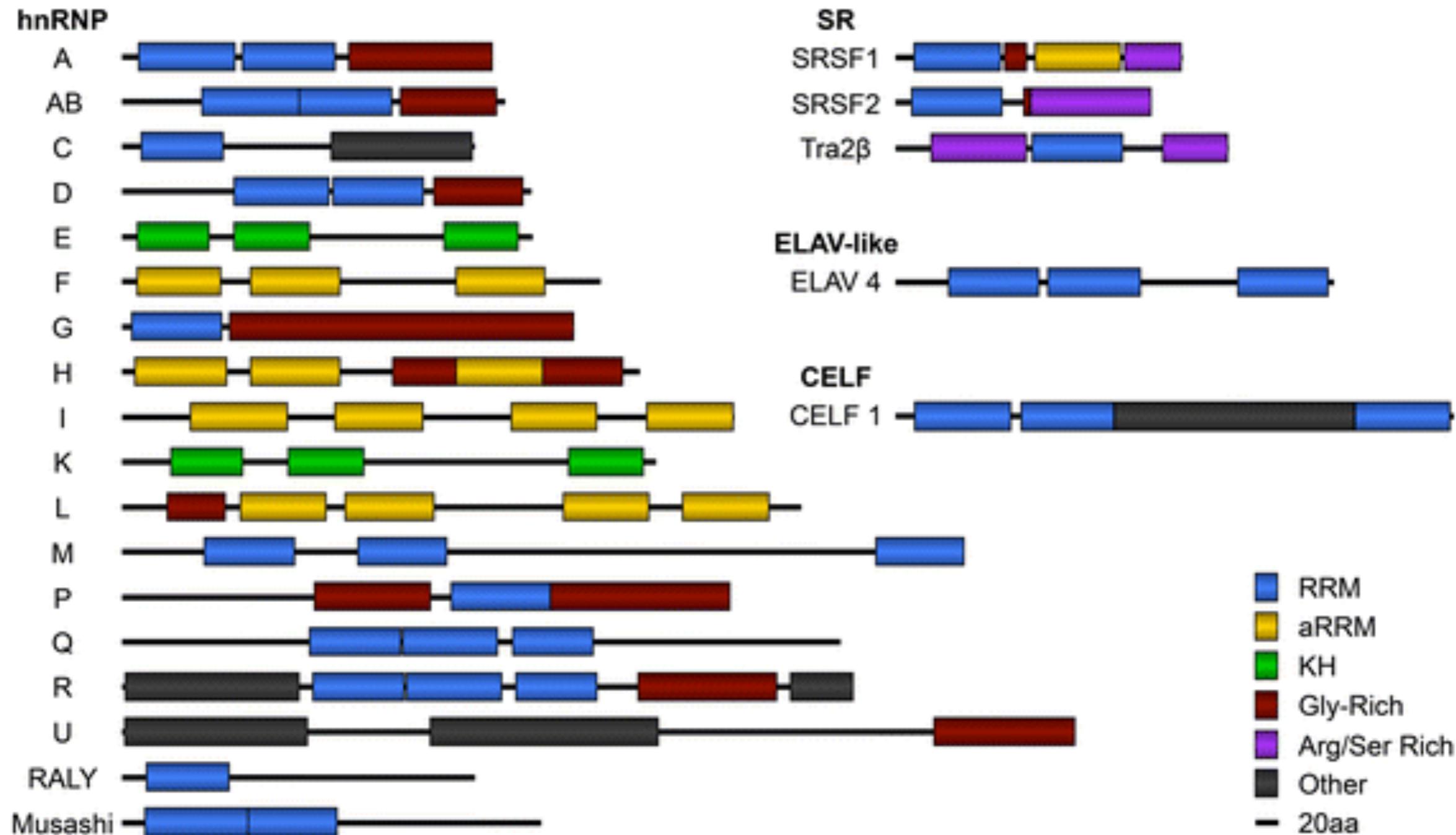
“Classical” SR proteins



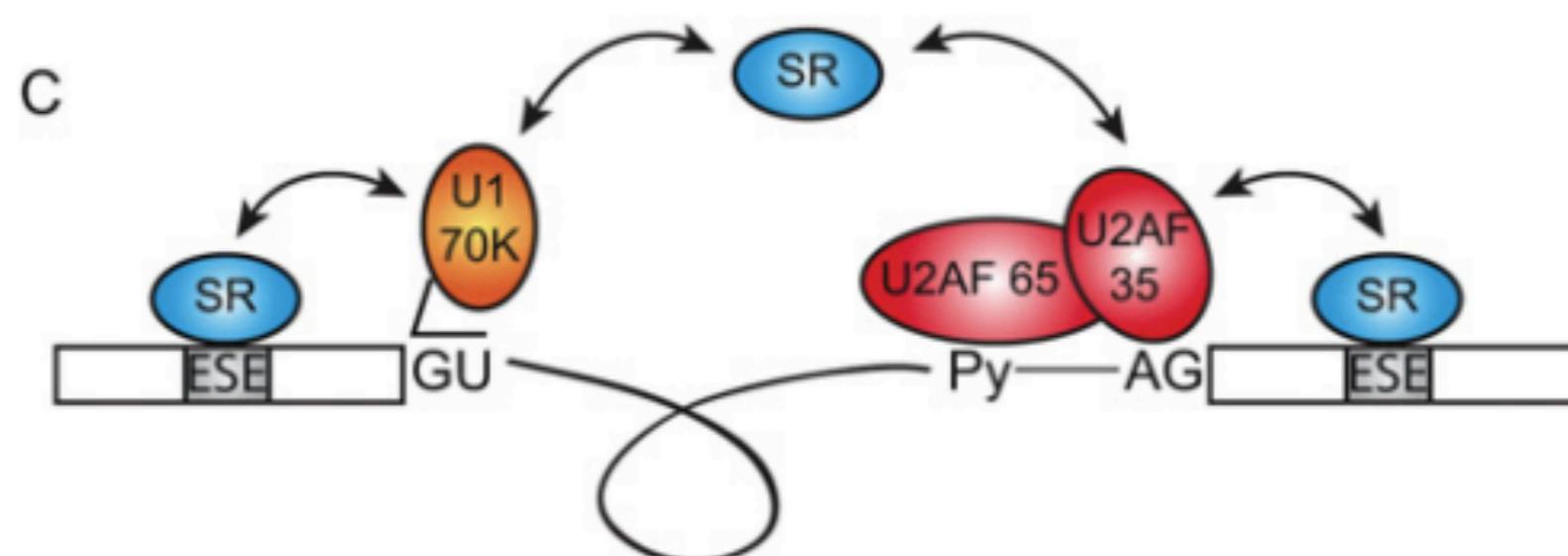
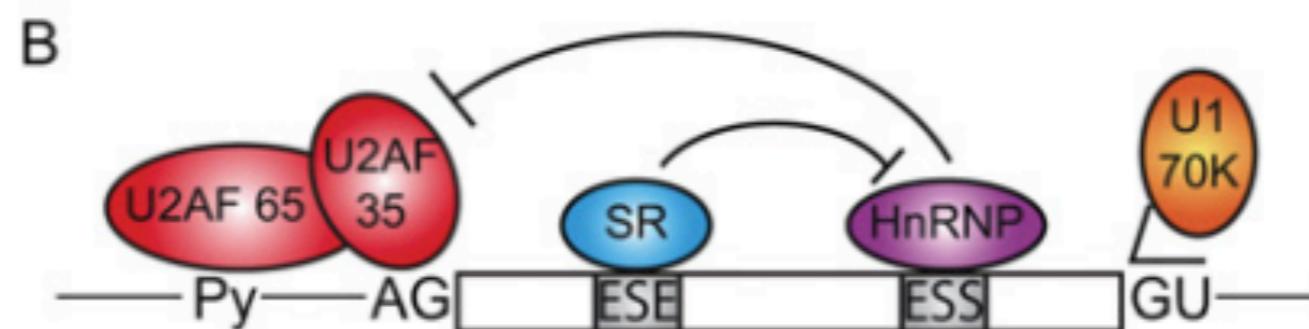
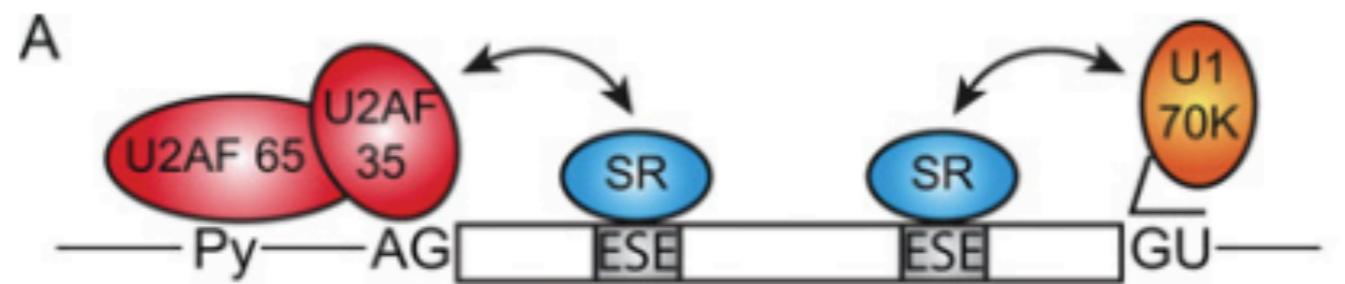
Additional SR proteins



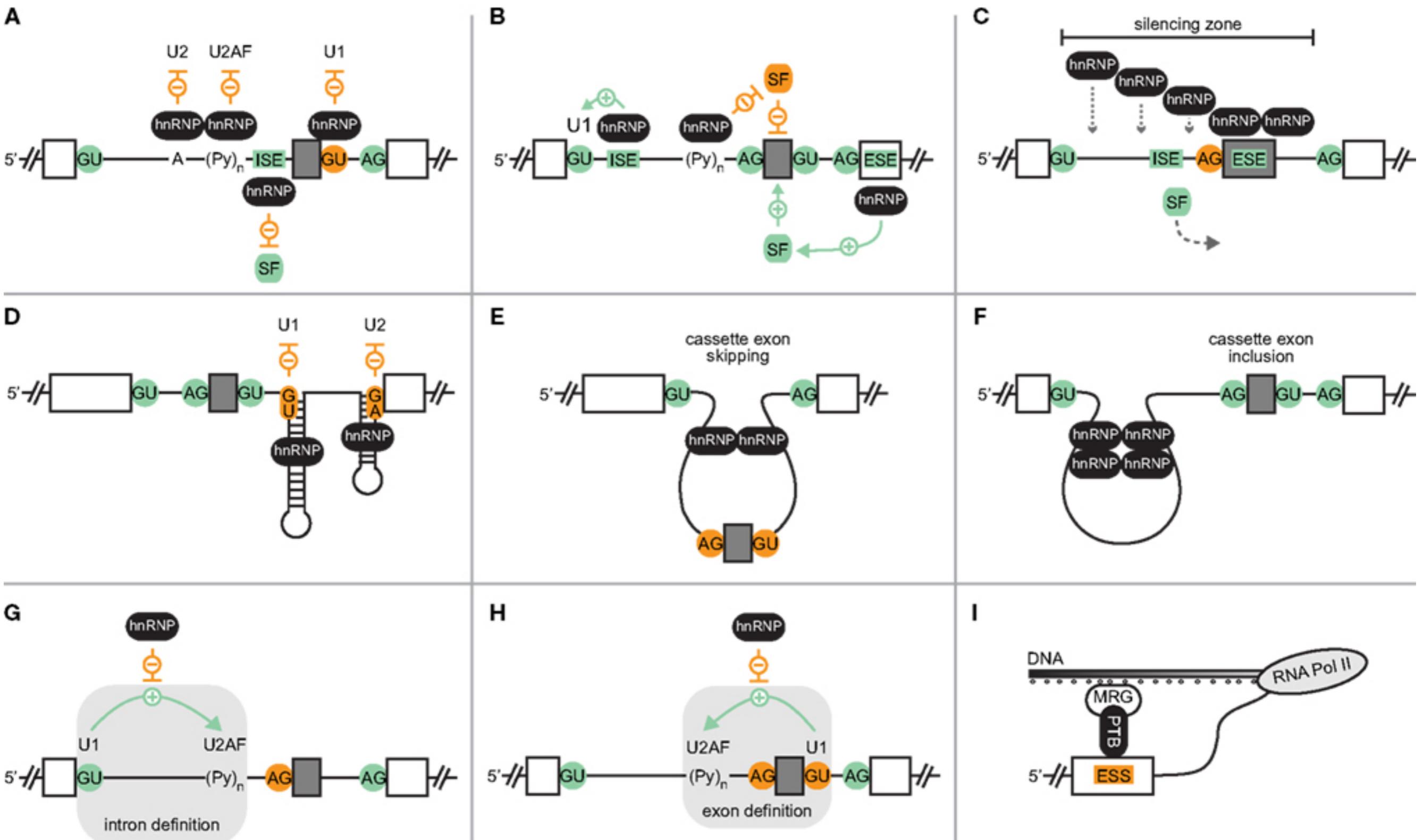
hnRNP proteins are more diverse in their domain structures



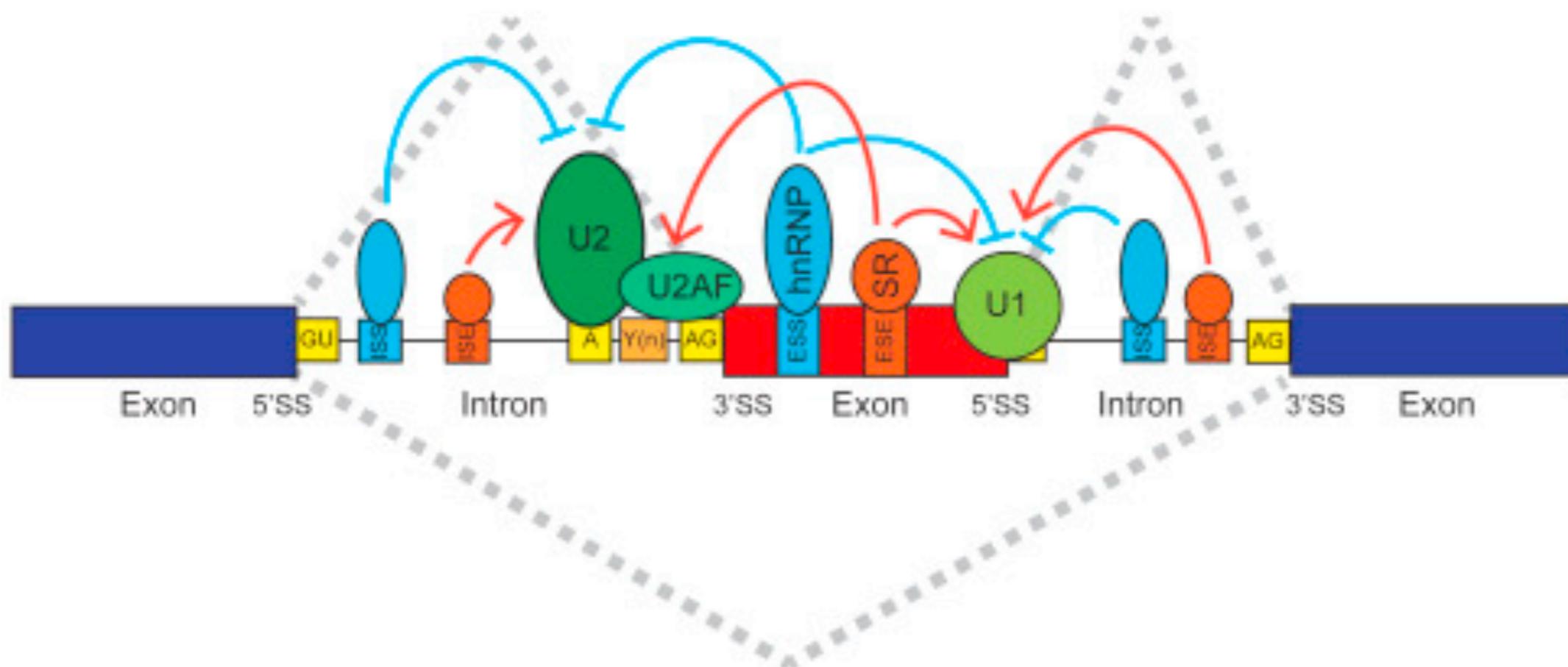
Mechanisms of splicing activation



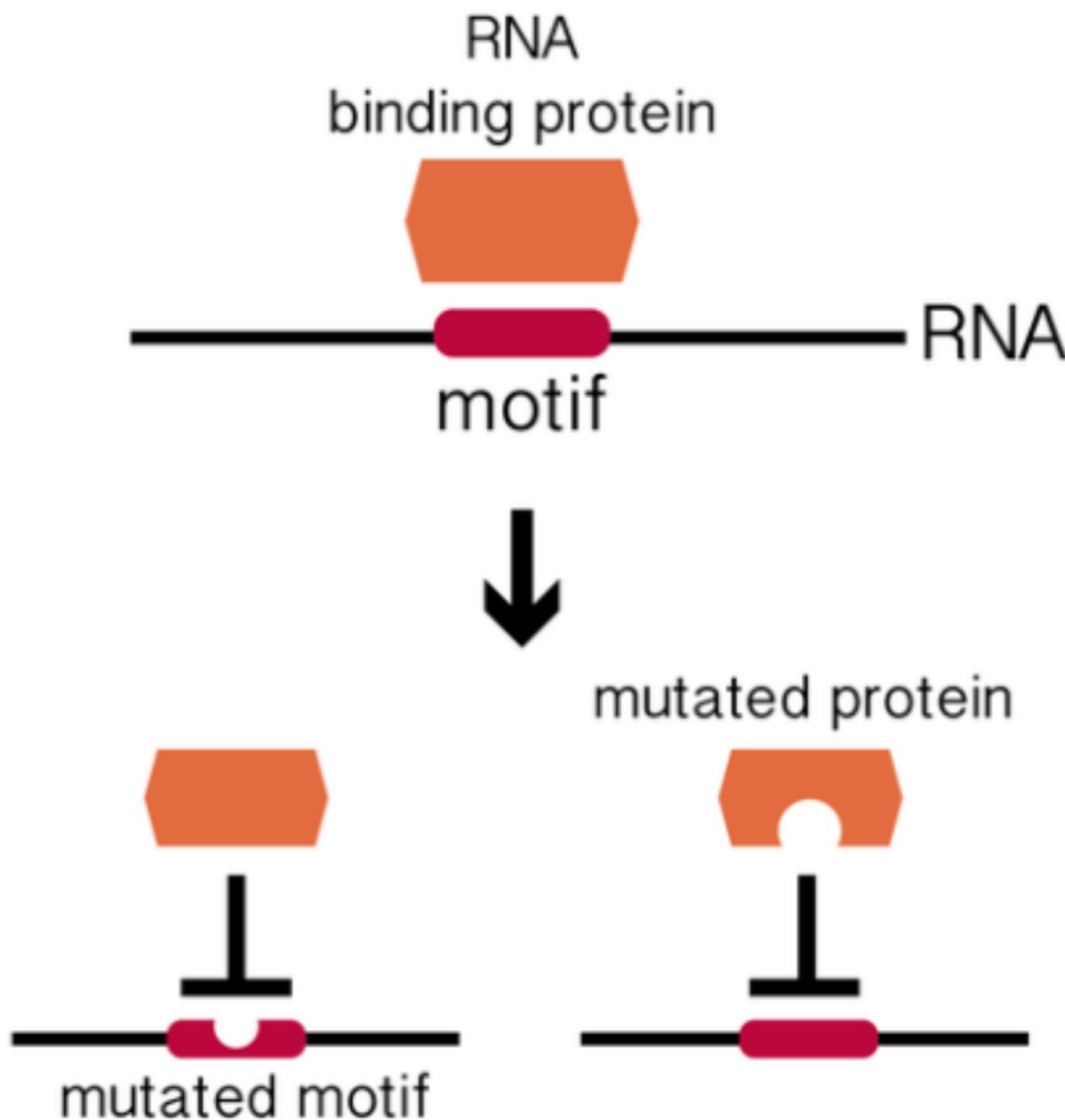
Mechanisms of splicing repression



Alternative splicing is regulated through the activity of specific RNA sequence elements

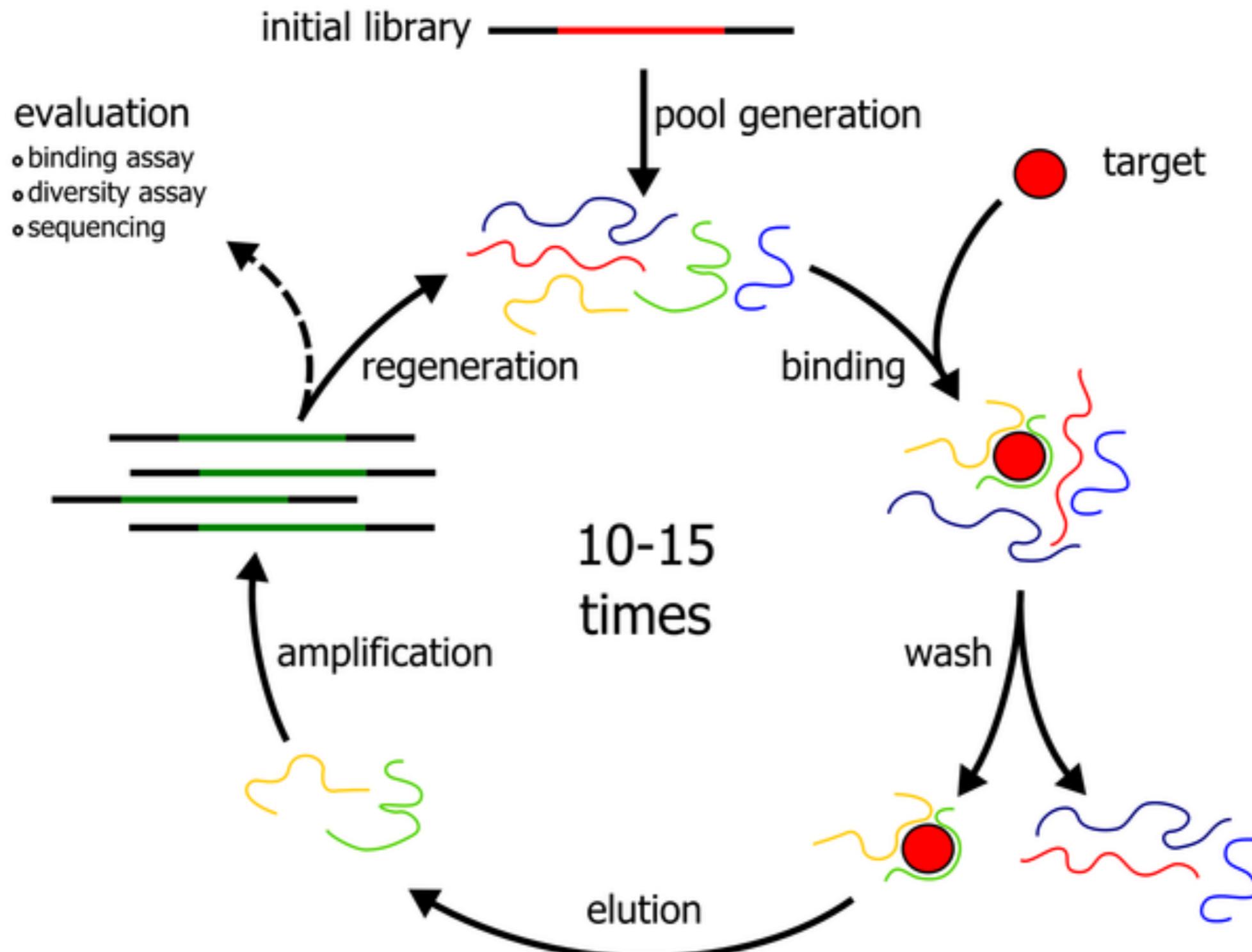


RNA-binding proteins (like SR proteins and HNRNPs) often prefer specific RNA sequences

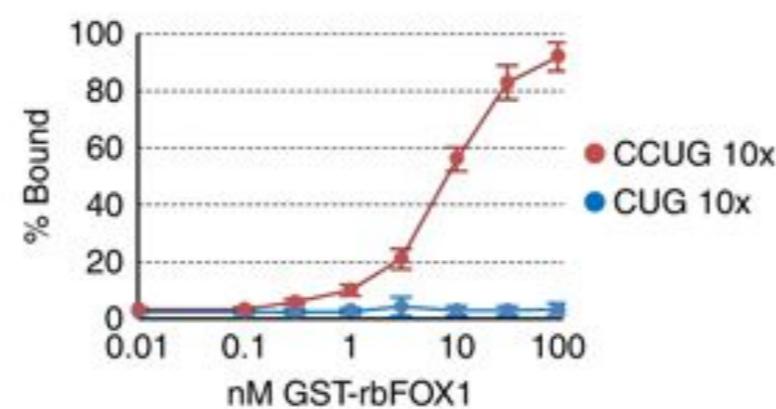
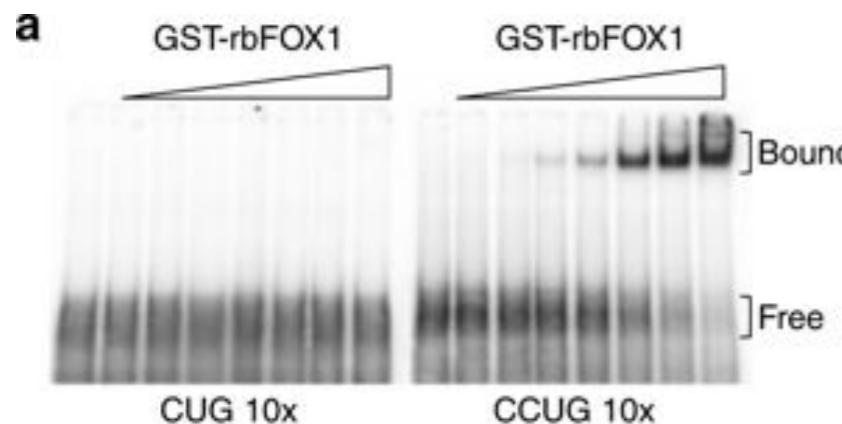


How do we find out what these motifs are?

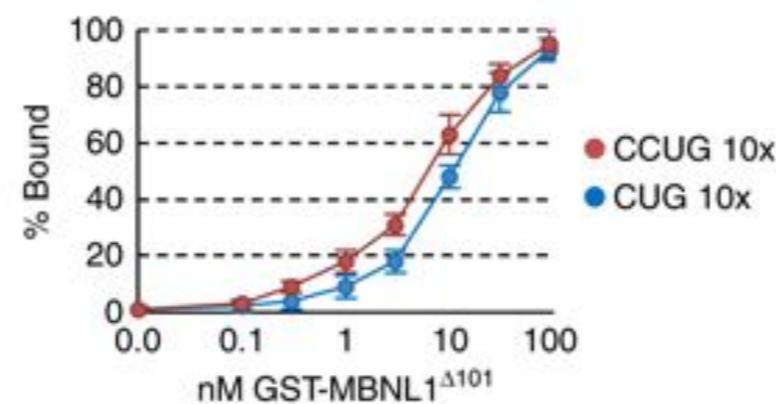
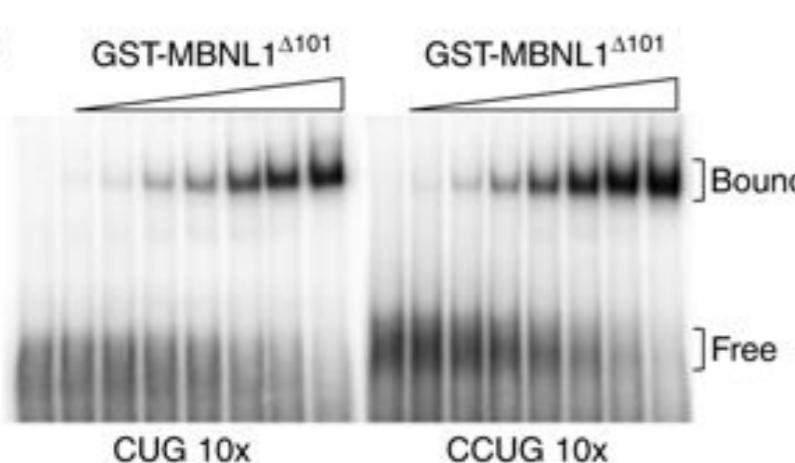
Identification of RBP-bound motifs with SELEX



Identification of RBP-bound motifs



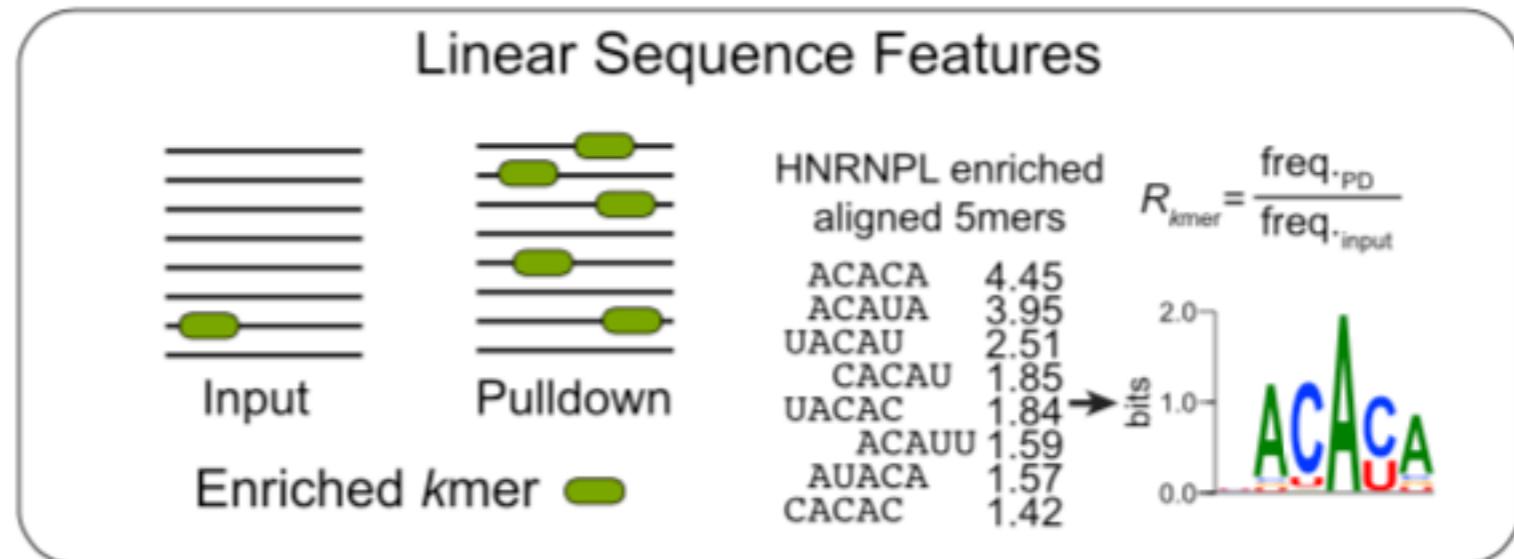
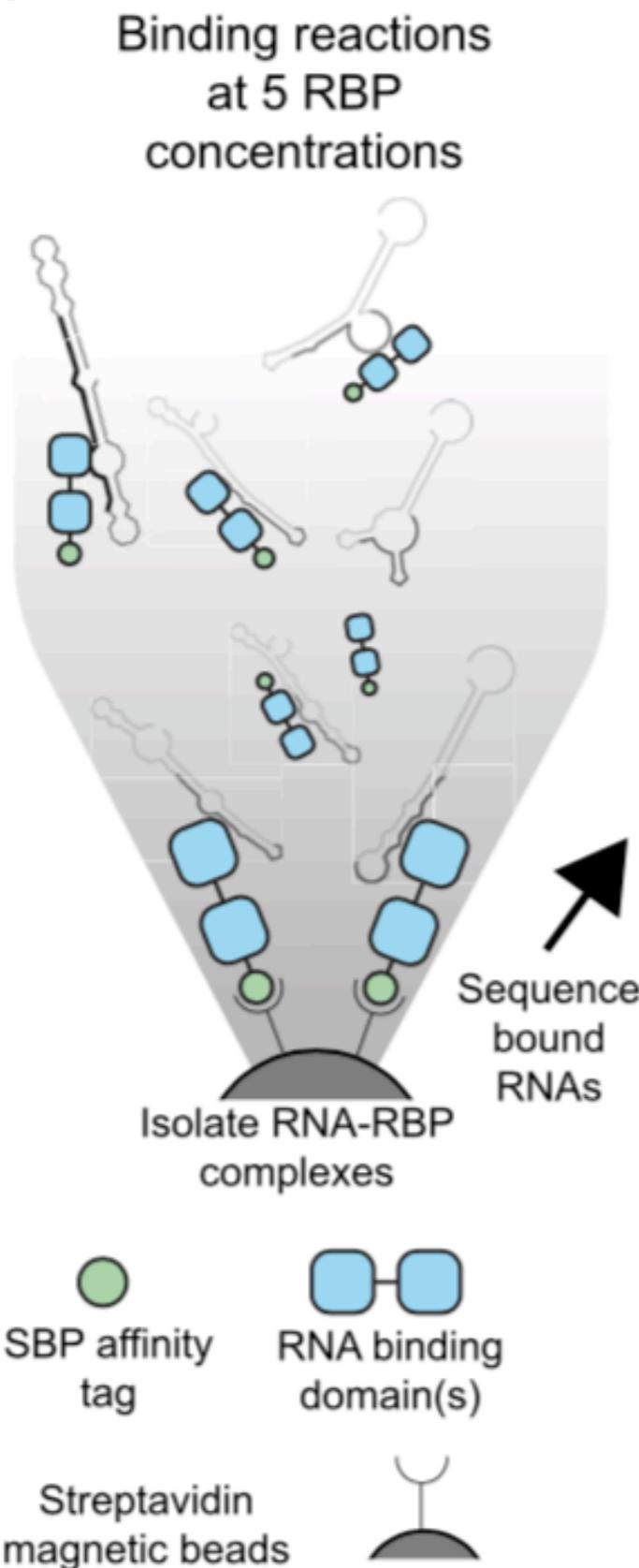
UGCAUG



UGCU / CGCU

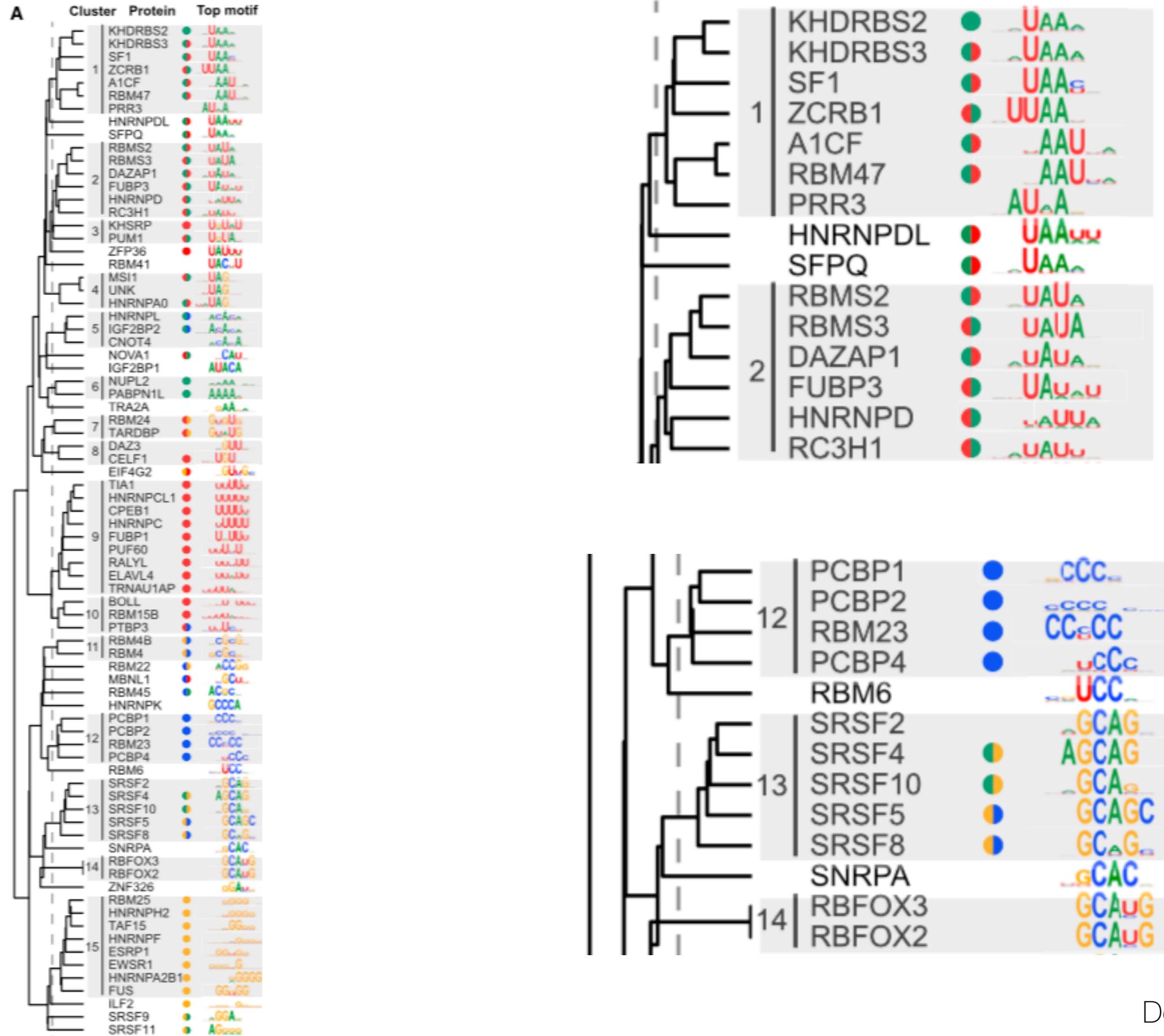
Identification of RBP-bound motifs with RBNS

A

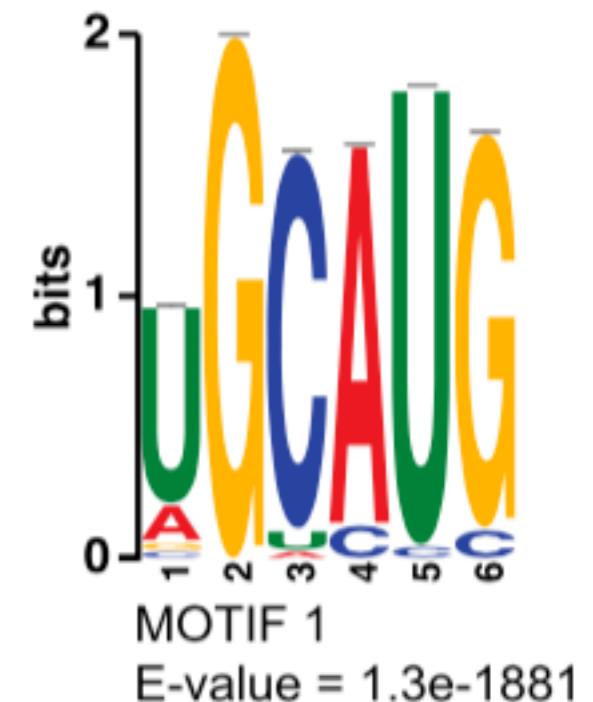
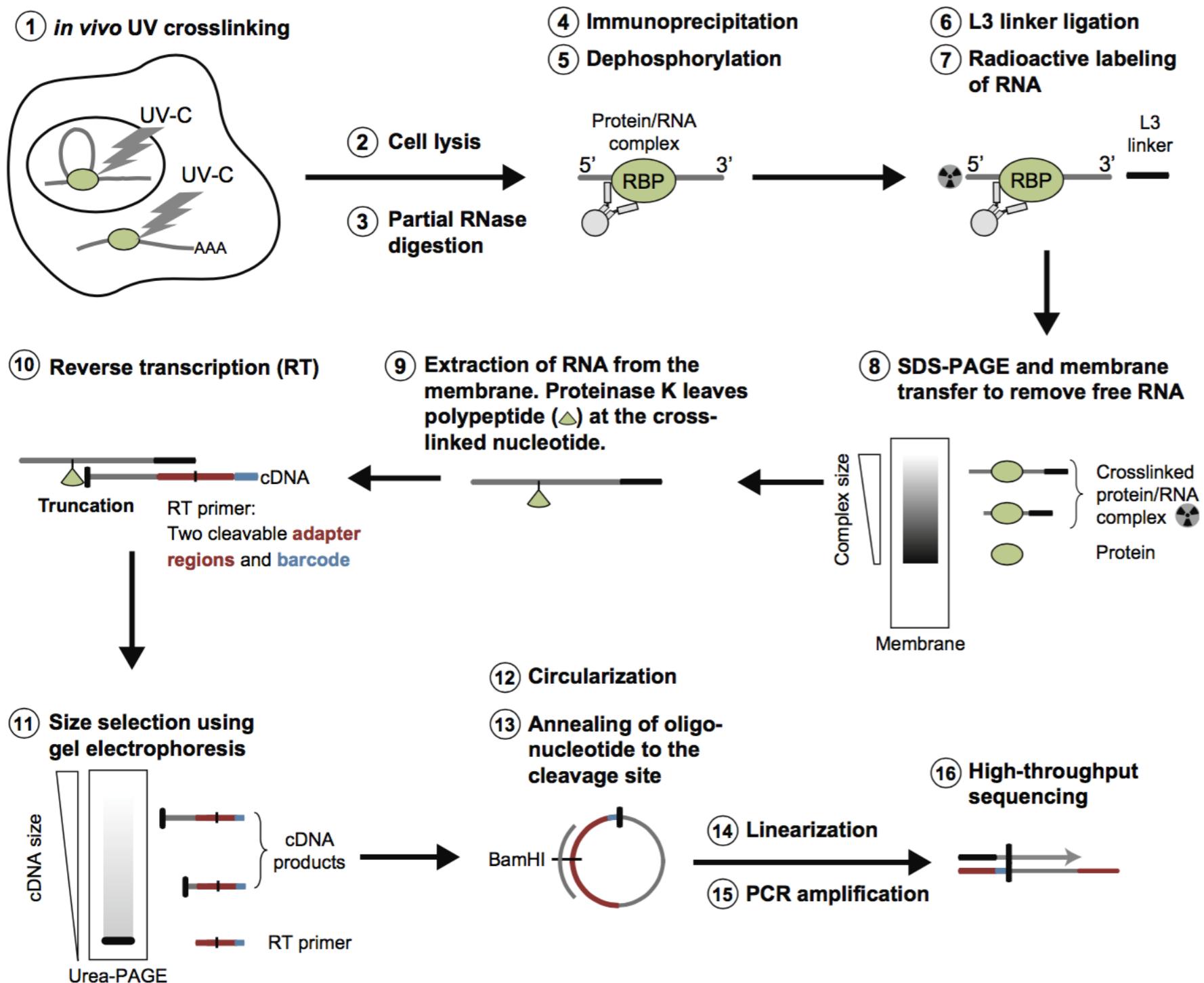


This technique has been used to study the RNA-binding activity of ~100 RBPs

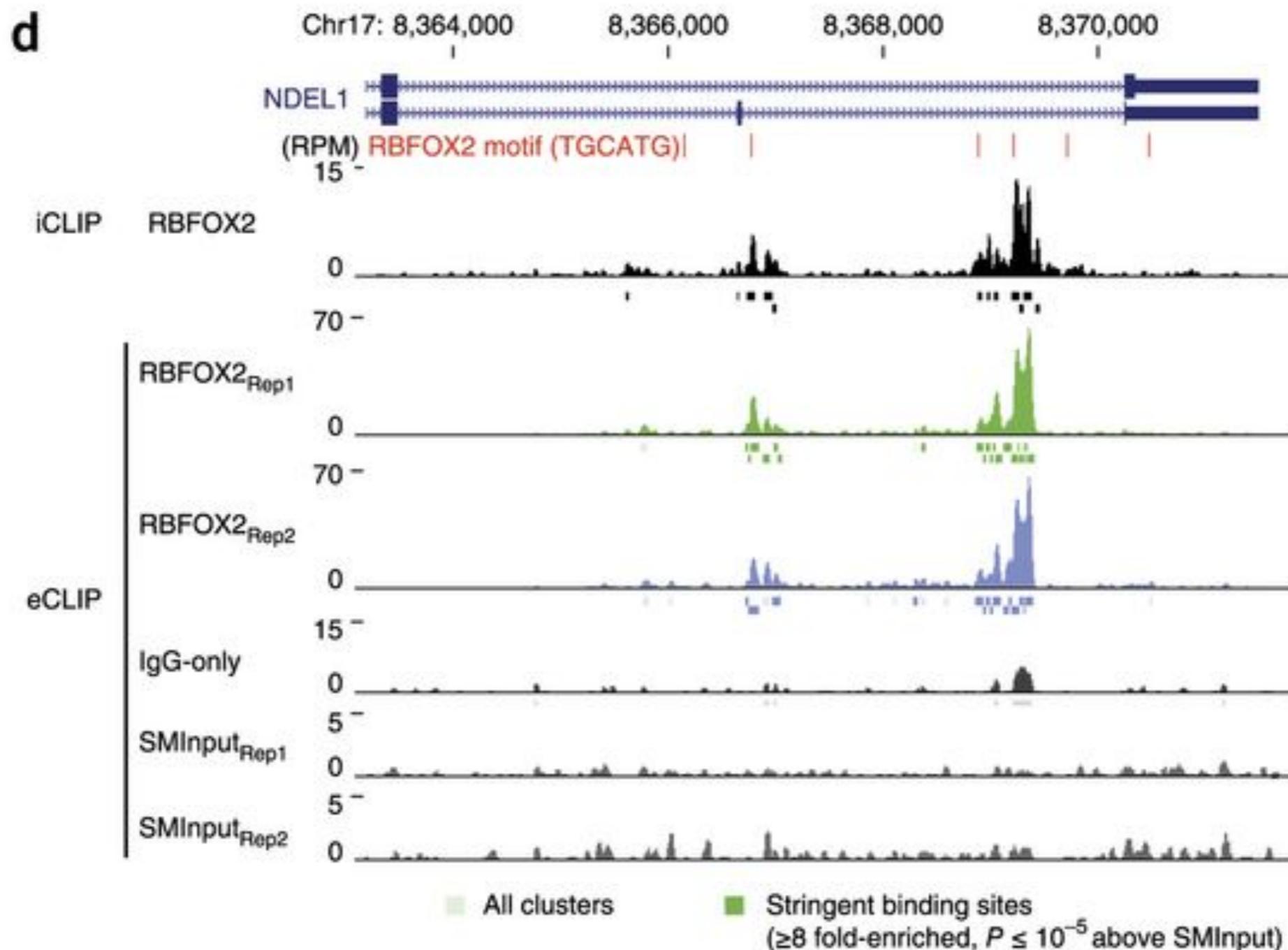
Identification of RBP-bound motifs with RBNS



Identification of RBP-bound motifs *in vivo*

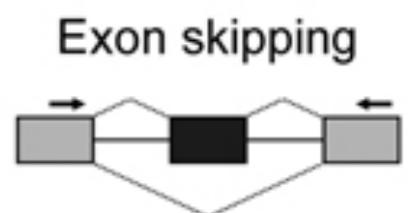
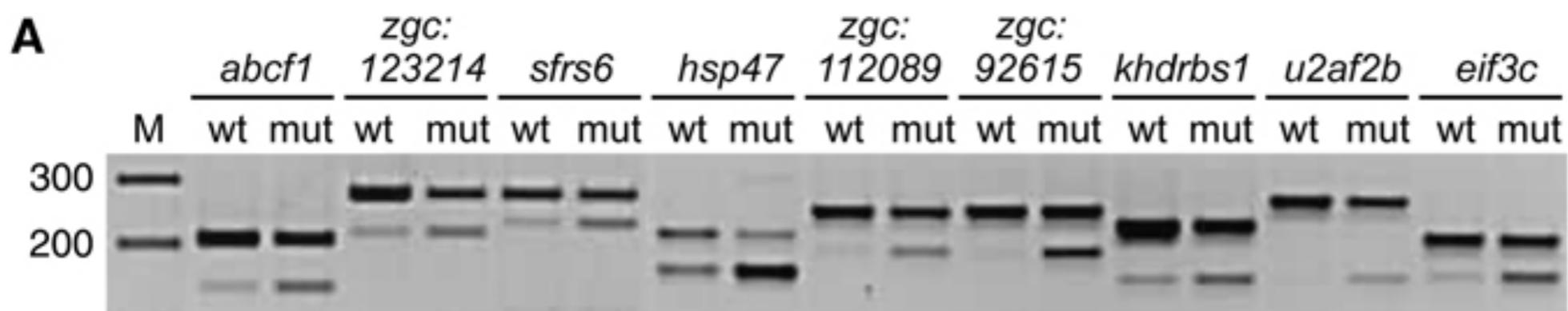


Identification of RBP-bound motifs in vivo

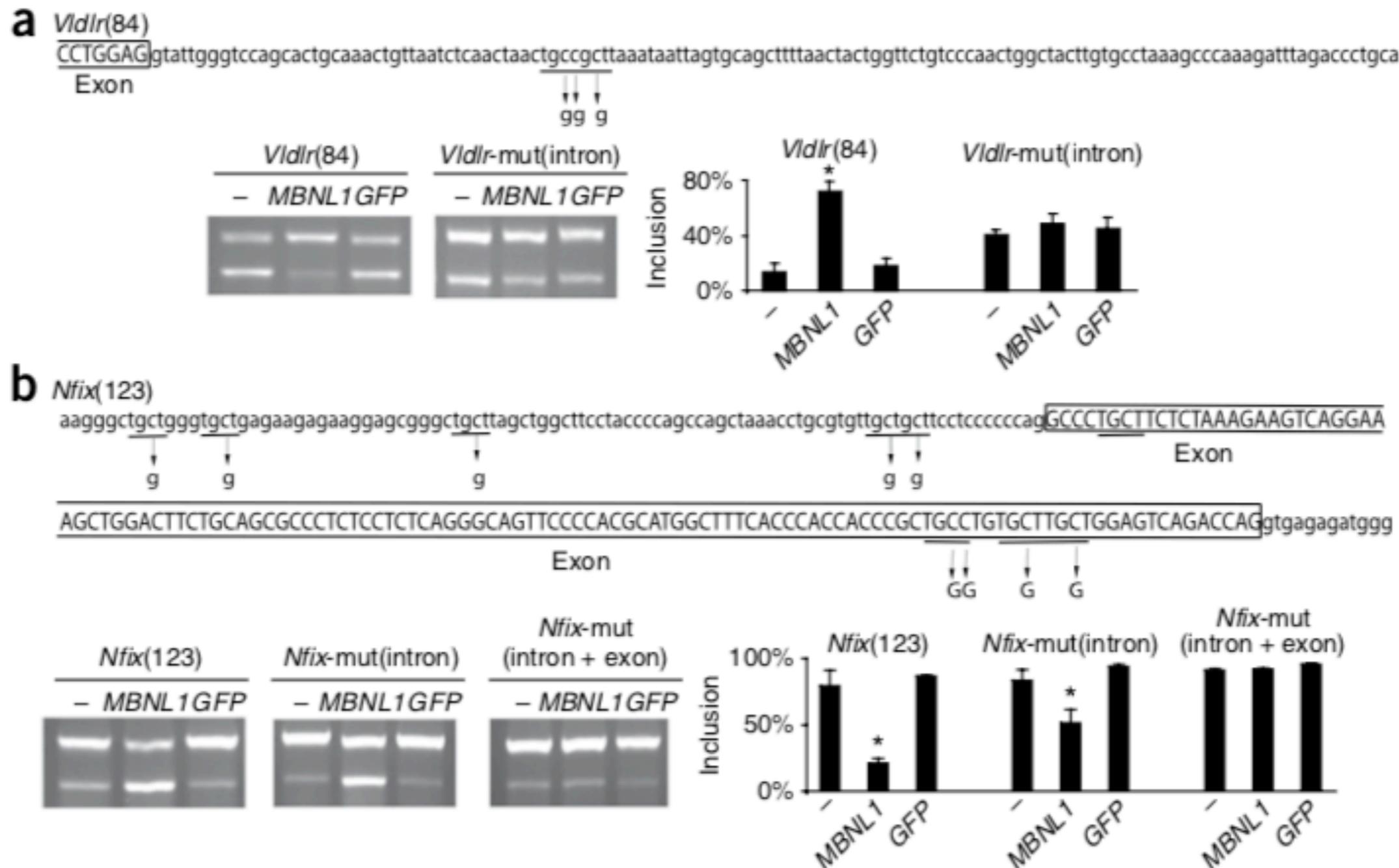


Why isn't every UGCAUG site bound?

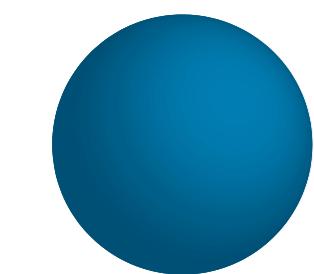
Monitoring the inclusion of single exons



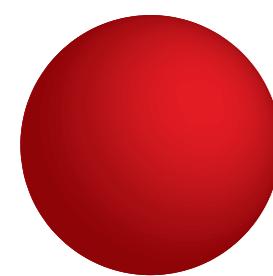
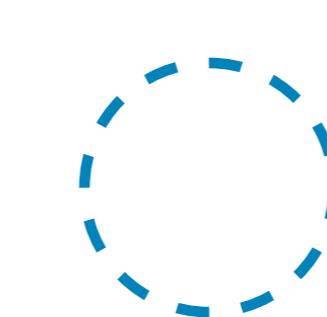
Assaying the effect of RBPs and motifs



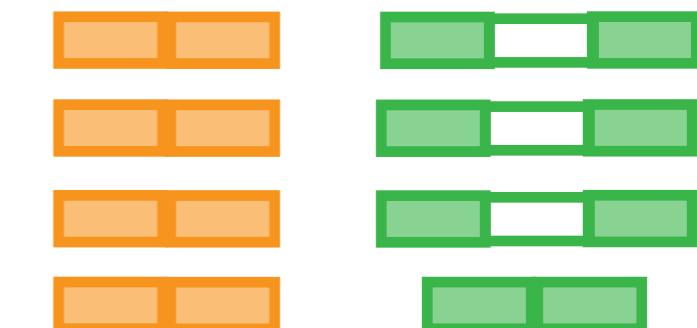
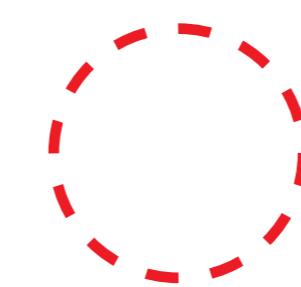
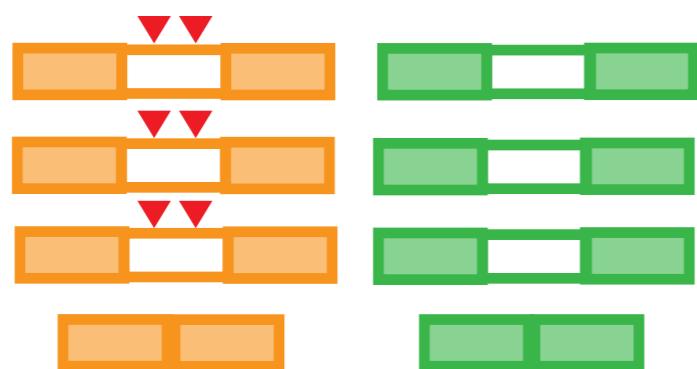
Using functional genomics to study RBPs and RNA processing



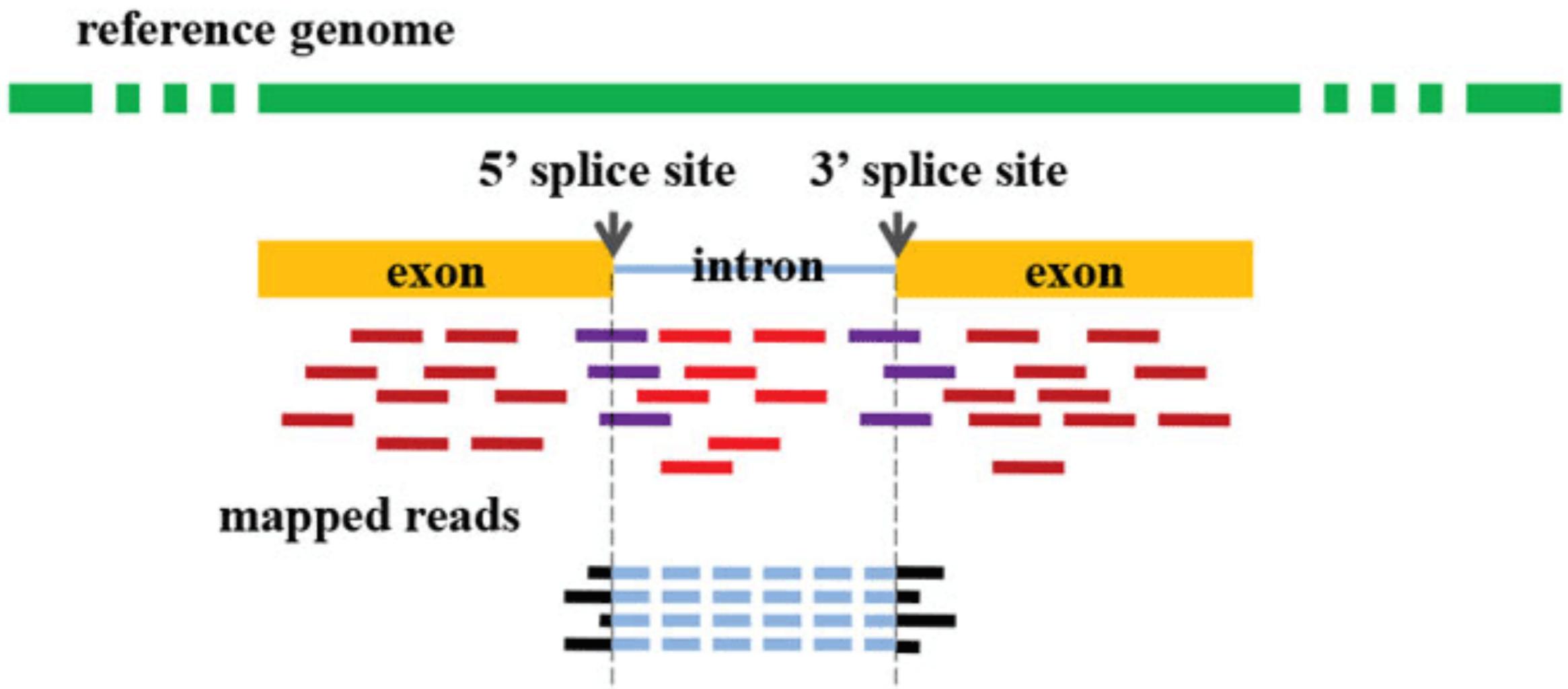
RNA stability



mRNA splicing



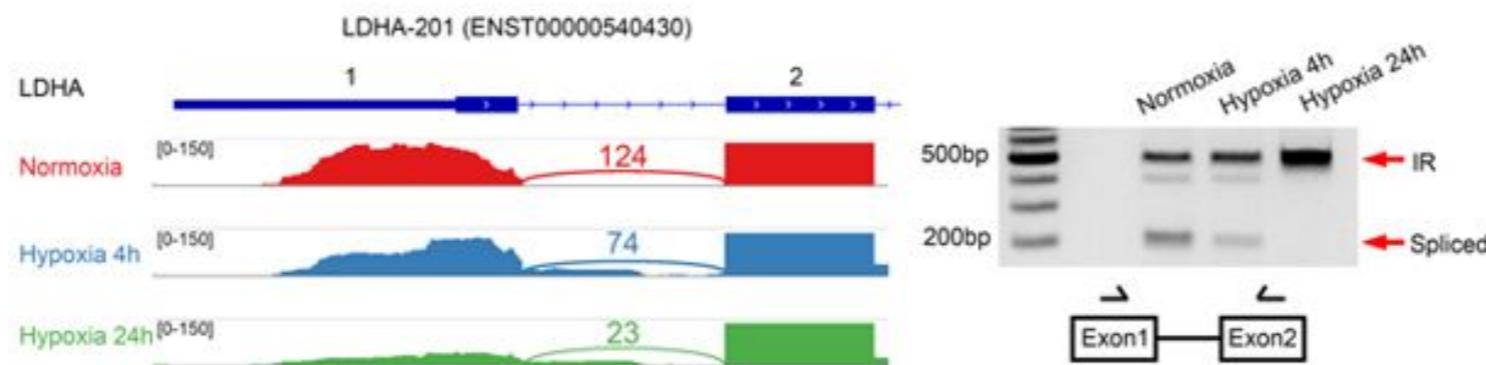
Quantifying alternative splicing with RNA-seq data



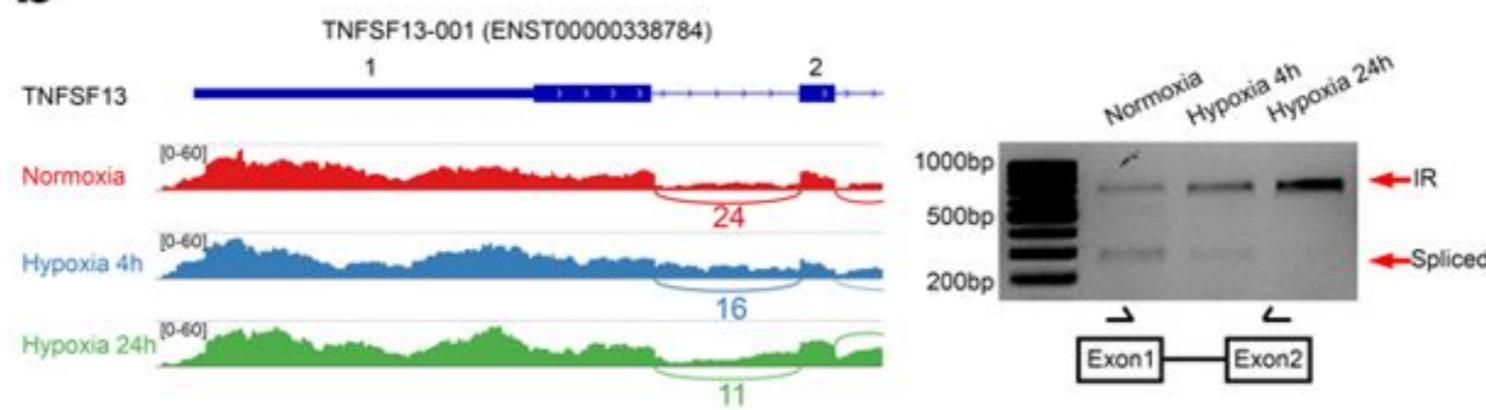
What are the relevant parameters that determine how informative an RNA-seq based alternative splicing experiment will be?

Quantifying alternative splicing with RNA-seq data

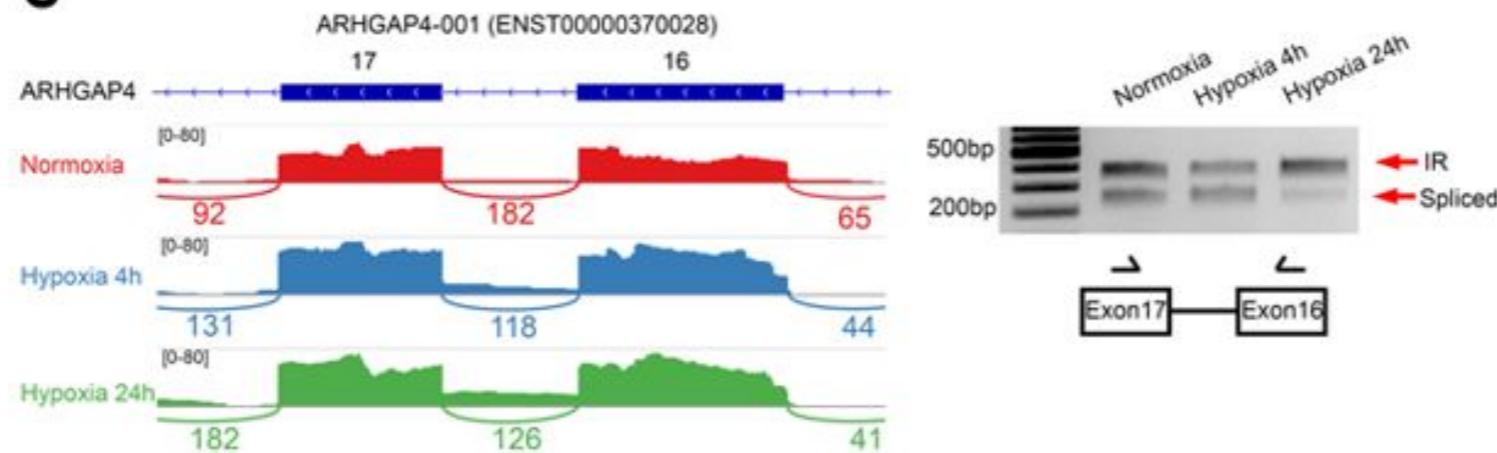
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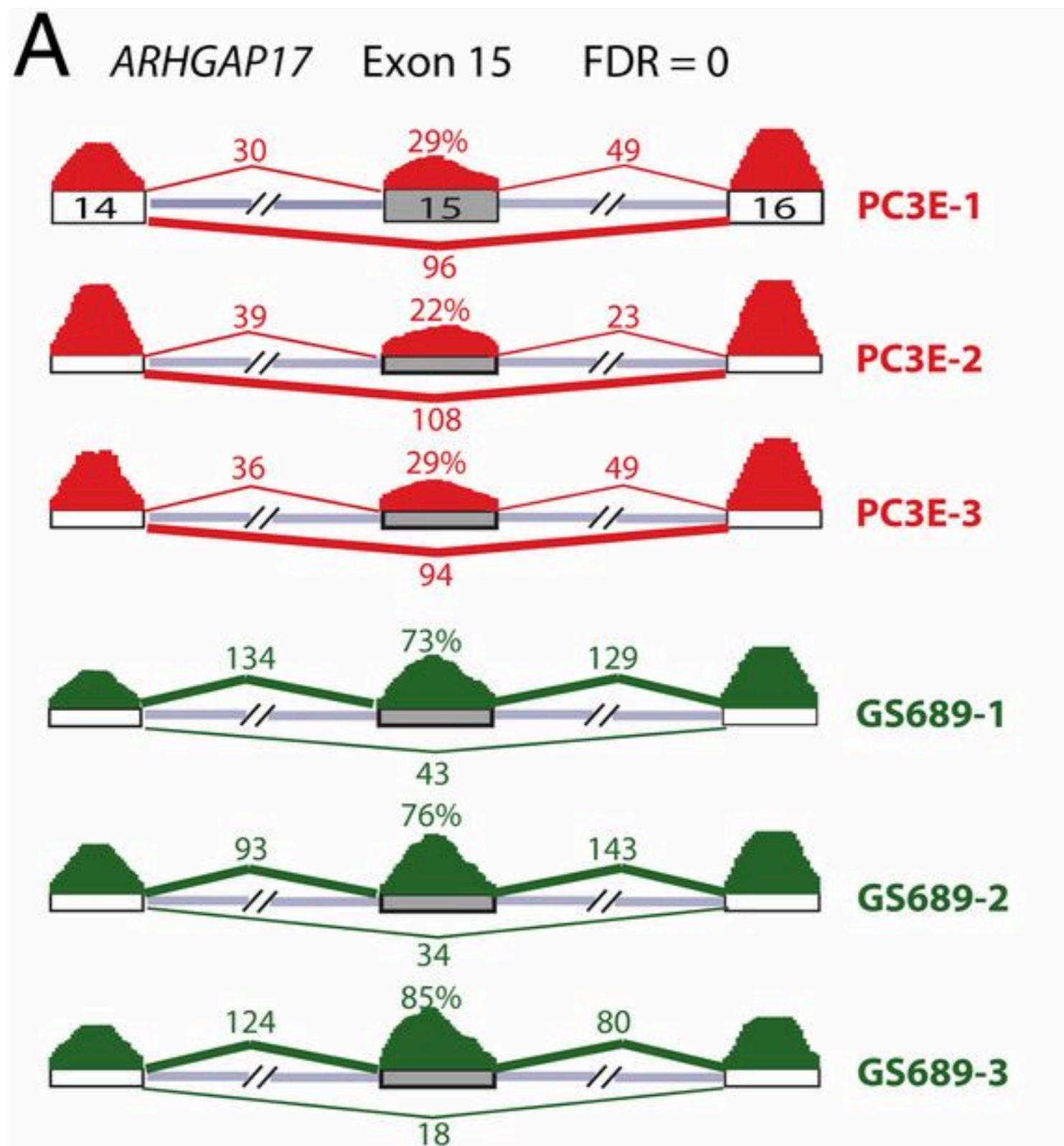
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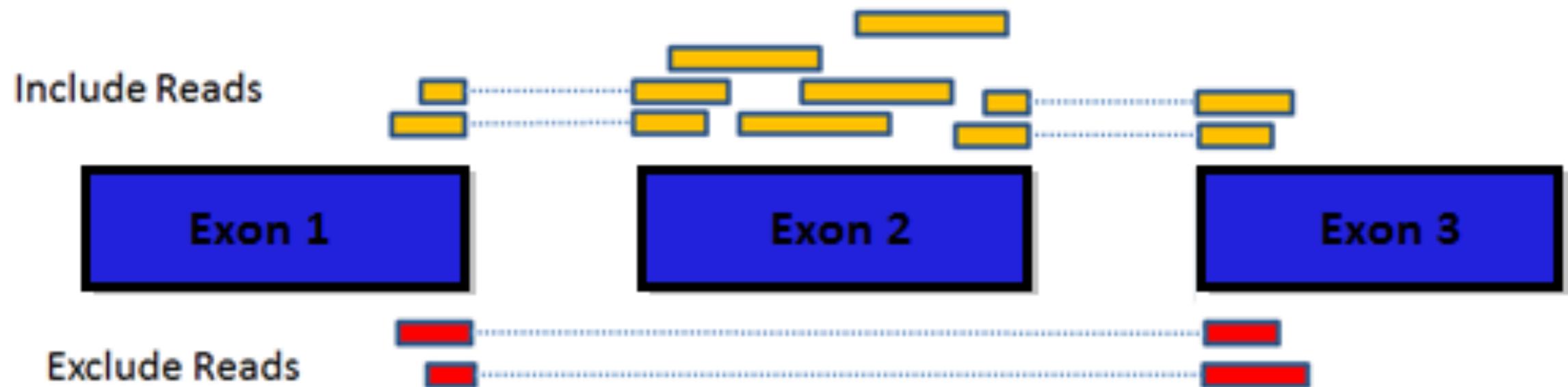
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Quantifying alternative splicing with RNA-seq data

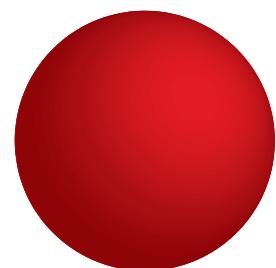


Quantifying alternative splicing with RNA-seq data

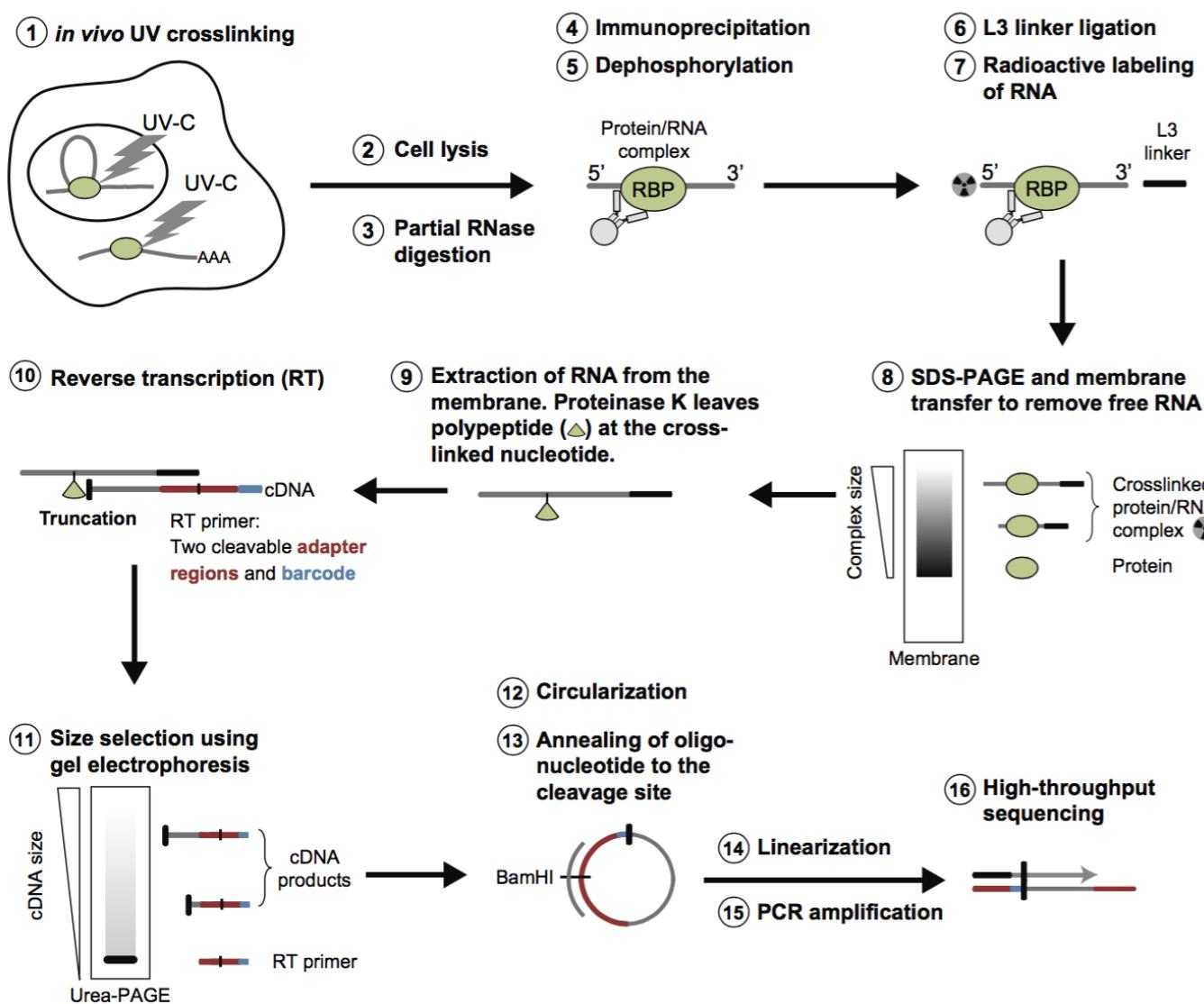
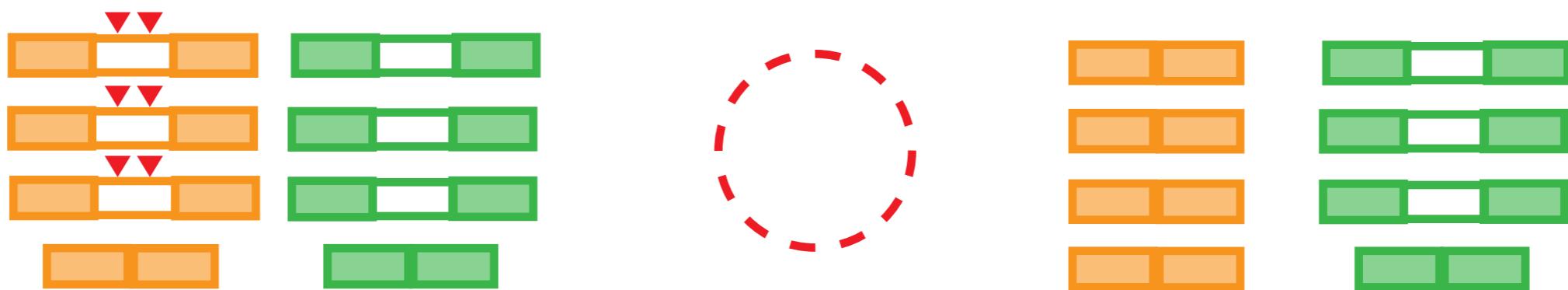


Exon Skip Percent Splice In (PSI): $8 / 10 = .8$ (Exon 2 included in 80% of transcripts)

Using functional genomics to study RBPs and RNA processing



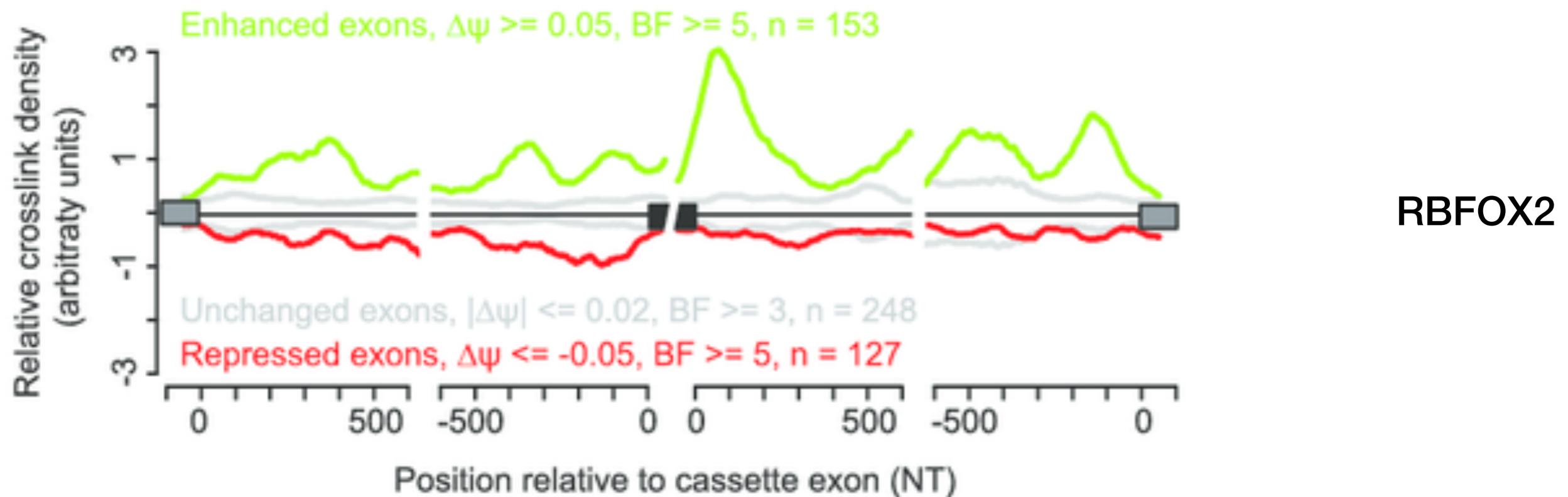
mRNA splicing



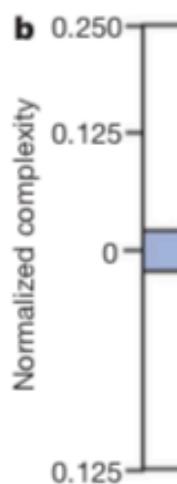
Combine knockdown-RNAseq and CLIP to determine the binding profile of an RBP near the exons it regulates to create an “RNA map”

Combining RBP knockdown, RNAseq and CLIP to learn about mechanisms of RBP action

A



RBFOX2



NOVA

Jangi et al (2014)
Licatalosi et al (2008)